The effects of the disposal of organophosphate and synthetic pyrethroid sheep dips on non-target organisms on farmland

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THE EFFECTS OF THE DISPOSAL OF ORGANOPHOSPHATE AND SYNTHETIC PYRETHROID SHEEP DIPS ON NON-TARGET ORGANISMS ON FARMLAND

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DECLARATION

The work contained in this thesis was carried out in the School of Biological Sciences at the University of Durham between September 1999 and February 2004. All the work was carried out by the author unless otherwise indicated. It has not been previously submitted for a degree at this or any other university.

Abstract

The Effects of the Disposal of Organophosphate and Synthetic Pyrethroid Sheep Dips on Non-Target Organisms on Farmland

L.J.Walker B.Sc. (Hons) Dunelm

The 1998 Groundwater Regulations required spent organophosphate and synthetic pyrethroid sheep dip to be disposed to farmland. The effects of dip disposal on soil invertebrates and the possible consequences for their bird predators were investigated on farms across Britain between 1999 and 2002.

A preliminary survey of dip disposal practice on 42 hill farms on, or adjacent to SSSIs that support breeding waders, exposed a wide variety of practices and considerable deviation from the recommended procedures in many cases. Paired disposal and control sites were sampled on a subset of the surveyed farms. Invertebrate abundance was estimated by taking soil samples, followed by Berlese extraction (or hand sorting for worms), pitfall trapping and suction sampling. Invertebrate densities on disposal sites were significantly lower than on control sites in 7 out of 15 cases and the multivariate analysis indicated significant effects of dip disposal on carabid, but not spider, species composition six months after application. Density reductions were greatest on areas that had been used for dip disposal over many years.

Plots were set up in a "Latin-square" design on two experimental farms, allowing comparison of the effects of the two insecticides at two dilutions under controlled conditions. The same sampling methods were used as on the farm sites and densities of all invertebrate groups, except linyphiid spiders and carabid beetles, were significantly reduced on the disposal plots on one or more sampling occasion after application. Soil surface invertebrates taken by suction sampling showed the most severe and consistent reductions.

A risk assessment suggests that spring disposal could compromise upland wading chick survival. However, the current scale of dip disposal in Britain does not pose a threat to whole bird populations.

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In the lab Astrid, Gillian, Ed and Dorothy brightened each day and were always ready with new ideas and much needed camaraderie. Friends, colleagues and staff at Van Mildert College were also a great source of strength throughout my studies. Special thanks are due to Laeti Vedrenne and Dr Daniel Fowler who rescued me on many occasions and James Smith who was a great help with all aspects of the Ph.D.

Finally, my parents have always been inspirational and unstinting in their support and I dedicate this work to them.

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1. INTRODUCTION

This study investigates the effects of the disposal of spent organophosphate and synthetic pyrethroid sheep dips on non-target organisms on farmland. The impact of pesticides on non-target invertebrates has been reviewed many times (Theiling and Croft, 1989) and research into the effects of pesticide poisoning on birds is increasingly comprehensive (Hall, 1987). However, the potential risks of dip disposal on areas of upland Britain that have not previously been exposed to pesticides on such a scale are hitherto unexplored.

Farmers have battled to protect their crops and livestock from insect pest species for many centuries. Early pesticides, including vinegar, soap and pyrethrum from the flowers of *Chrysanthemum cinerariaefolium*, were supplemented in the 1800s by very toxic chemicals such as arsenic and cyanide. However, until 1939 the most effective chemicals were prohibitively expensive for most farmers, who consequently welcomed cheaper, mass produced, synthetic insecticides (Mellanby, 1967). The advent of organochlorine pesticides in the 1940's was heralded as an answer to the growing problem of supplying good quality, cheap food to the increasing world population and since then the agrochemical industry has introduced huge range of biologically active chemicals' (Eke *et al.*, 1996; Somerville, 1990).

However, the substantial benefits of the use of pesticides may be accompanied by major disadvantages, predominantly the detrimental effects to non-target organisms (Somerville, 1990). When Rachel Carson first published her book 'The Silent Spring' in 1962, she raised awareness of the increasing environmental and public health problems associated with pesticide use and prompted much greater public scrutiny of the agrochemical industry. The evidence provided by Carson of the devastating effects of pesticides on non-target species of insects, birds and mammals, including man, was followed by further research and legislative measures to curb the pollution and improve farming practices. These include Part III of the Food and Environment Protection Act 1985 (FEPA) and The Control of Pesticide Regulations 1986 (COPR). However, despite increasing knowledge from the interim years of research, there are still substantial undesirable environmental and socio-economic costs of world-wide pesticide usage (Harvey, 1997; Pimentel, 1992).

Studies into the effects of pesticides on soil fauna reveal a highly complex problem (Wallwork, 1976). Increasing numbers of studies have shown detrimental effects of exposure to pesticides to non-target beneficial invertebrates, including predatory invertebrates such as carabid beetles and lycosid spiders, pollinating insects such as bees and many species which contribute to bird diets (Cole and Wilkinson, 1985; Hill, 1985; Dempster, 1968a,b; Moreby and Southway, 1998; Moreby *et al.*, 2001; Sotherton, 1989; Vickerman and Sunderland, 1977).

Even relatively short lived periods of pesticide toxicity in the soil can result in large declines in invertebrate densities and in taxa with a restricted breeding season, such as Carabidae and Staphylinidae, recovery is slow (Wallwork, 1976). Further complications occur when repeated applications result in accumulation of toxic compounds in the soil and predator/prey interactions can be severely disrupted (Wallwork, 1976; Burn, 1989; Shires, 1985). Carabidae, for example, have shown a delayed response to pesticide application, attributed to reduction in prey availability impacting indirectly on the carabid community (Burn, 1989). Absolute removal of all prey items does not usually occur in such cases but as a positive relationship exists between food availability and egg production in predator species fecundity and recruitment can be impaired (Burn, 1989; Mols, 1979; Murdoch, 1966).

In cases where one species of invertebrates is directly depleted by pesticide application others may be unaffected and the balance within the community is altered (Wallwork, 1976). Studies on the effects of DDT on predatory mesostigmatid mite and collembolan prey species showed that while the mite populations were severely depleted the Collembola were relatively unaffected and their populations increased dramatically post application (Edwards, 1964,1965). The predatory invertebrates remaining after pesticide application may increase in number as a result of exploitation of the elevated numbers of available prey, as found by Dempster (1968a,b) where populations of the carabids *Nebria brevicollis* and *Trechus quadristriatus* increased when the density of Collembola rose in response to a pesticide induced reduction of other predator species. Carabid beetles are an important component of the diet of wading birds (Baines, 1988) and therefore a change in prey ratio can also affect vertebrates higher in the food chain by increasing or, more often decreasing invertebrate food supply.

The initial links between repeated sprayings of the organochlorine pesticide DDT and the reduction in many bird populations (Carson, 1962) have subsequently been echoed in later studies of both DDT and other pesticides (Brewer *et al.*, 1993; Hall, 1987). On areas subjected to a pesticide regime insectivorous birds are at risk of the direct effects of insecticide poisoning, delayed effects due to storage of the poisons in metabolically inactive body tissues that can cause mortality upon release, and indirect effects from a reduction in food supply (Evans, 1990; Moreby and Southway, 1998; Brewer *et al.*, 1993; Hall, 1987). Relatively recently organochlorine pesticides have been detected in the testes of male grassland nesting passerines in studies in North America, with significantly higher levels in insectivorous birds than omnivores and granivores and possible endpoint effects of contamination such as hormone disruption (Bartuszevige *et al.*, 2002). A range of insecticides including organophosphates and synthetic pyrethroids have been found to directly impair the health of tree swallows (*Tachycineta bicolour*) by inducing anaemia and over stimulating the immune system and the contaminants have been measurable in the swallow eggs (Bishop *et al.*, 1998). Synthetic pyrethroid insecticides have also been found responsible for Blue Tit mortality and lowered breeding success through indirect effects via shortage in prey availability (Pascual and Peris, 1992) and the severe decline in numbers of farmland birds in Britain, has been attributed to reduction in food supply due to the use of a variety of insecticides (Sotherton, 1989; Moreby and Southway, 1998). The above studies are just a few of the many possible examples that provide evidence of the ongoing effects, in modern day farming, of pesticide poisoning of non-target organisms and the continuing research into the problem.

Despite the associated environmental problems, pesticide usage remains widespread as it is an effective method of pest control in crops and livestock. This is particularly the case for hill sheep farmers who must use some form of insecticide to protect their flocks from potentially lethal insect pests. Sheep dipping, involving full immersion of the sheep, has traditionally been used by farmers to control harmful insect pests. The two types of pesticide used for sheep dipping are organophosphates and synthetic pyrethroids. Organophosphate (OP) insecticides inhibit the enzyme cholinesterase thereby affecting the nervous system (Mellanby, 1967; Tomlin, 1997). Organophosphates are known as 'stripping dips' as the active ingredient is removed from the dipwash during sheep dipping and remains in the wool (Meat and Livestock Commission, 2000). The active ingredient must therefore be regularly replenished during the dipping process. Diazinon is the active ingredient commonly used in many organophosphate based sheep dips. It is a non-systemic insecticide and acaricide and is known to control a wide variety of sucking and chewing insects and mites (Tomlin, 1997). Synthetic pyrethroid (SP) insecticides also affect the insect nervous system (Miller and Salgado, 1985) and are known as 'non-stripping dips' in which the concentration of active ingredient remains the same throughout the dipping process (Meat and Livestock Commission, 2000). A typical example widely used in sheep dips is Cypermethrin, which is non-systemic and exhibits contact, stomach and anti-feeding action and also controls a wide range of insects (Tomlin, 1997).

One of the merits of sheep dip is that it treats a broad range of pests including blowfly strike, ticks, lice and, very importantly to the hill sheep farmer, sheep scab mites. Blowfly strike results in loss of appetite and condition as the infected animal is literally eaten alive and can result in death in as little as three days and lice cause severe skin irritation and ticks transmit debilitating diseases including tick borne fever (Cooper and Thomas, 1983). However, in 1998 members of the Moredun Foundation, one of the world's leading animal health and welfare institutes, identified sheep scab as a major threat to sheep health and welfare (Meat and Livestock Commission, 2000). Sheep scab is highly contagious and is a predominant issue where farmers share common grazing rights to rough grass and moorland, where sheep that may have been missed in the gathering at dipping time can infect 'clean' flocks and many flocks can come into contact with each other. The highly prolific scab mite, *Psoroptes ovis*, takes 14 days to mature from egg to adult, adult females can live for up to 50 days depositing one to two eggs per day and the mite can remain infestive off a host for fifteen to sixteen days (Meat and Livestock Commission, 2000). Scab mites cause highly irritating lesions due to allergic dermatitis that causes the sheep to rub and gnaw the infected area. There are few early symptoms of scab but in later stages severe crusting over large areas and wool loss can culminate in secondary bacterial infection, emaciation and eventual death in extreme untreated cases. Under the Sheep Scab Order 1997 it is a criminal offence to fail to treat sheep visibly affected by sheep scab and all other sheep in the flock. In addition to the welfare and legal issues an infestation has economic effects due to reduced fleece and leather quality, reduced conception rates and poor growth in young animals. For these reasons the use of pesticides known to control the aforementioned insect pests and protect sheep flocks is an integral part of the farmers' annual regime.

Alternatives to traditional plunge or full immersion dipping include 'pour-ons' and relatively recently introduced 'injectables'. Pyrethroid pour-ons involve pouring a measured amount of chemical along the back of each animal and uses less chemical than full immersion dipping. They are widely used to treat ticks and lice but are ineffective against the sheep scab mite (Meat and Livestock Commission, 2000). Systemic endectocides, such as doramectin and ivermectin, administered by injection, can kill both scab mites and gut worms but may not control blowfly strike. Therefore sheep dipping remains the most popular method of pest control, providing complete cover against a broad range of insect pests. However, a medium sized dip bath may contain 1500 litres of spent dip and traditional sheep dipping methods produce large amounts of waste pesticide at the end of the dipping process. The disposal of the spent sheep dip gives rise to significant environmental concern.

Prior to 1998, traditional dip disposal practices had included bucketing the pesticide out of the dip bath onto a small patch of ground closeby or down a nearby drain. Alternatively the plug was pulled out of the bottom of the dip bath to release the dip into the ground below. These practices resulted in frequent episodes of pollution in water courses during which aquatic invertebrates were eradicated from some areas and thousands of fish were killed over stretches of river in Britain. Data collated by the Tweed River Purification Board revealed incidents of pollution involving OP sheep dips affecting up to 3km stretches in the River Tweed between 1978 and 1988 (Virtue and Clayton, 1997). In Wales up to 45% of all pollution incidents in streams were attributed to sheep dip in 1998 (EA UK, 1998). Although they have a lower toxicity to mammals, SP sheep dips are more than a hundred times more toxic to aquatic life than OPs (EA UK, 1999; Pearce, 1997; Virtue and Clayton, 1997). In July 1995 a small spillage of dilute Cypermethrin waste dip in a tributary of the Slitrig Water killed 1200 fish and depleted the aquatic invertebrate population for 5km downstream (Virtue and Clayton, 1997). In April 1996 30km stretch of the river Caldew near Carlisle was practically stripped of all invertebrate life due to SP dip pollution (Pearce, 1997). During the late 1990's public concerns about the effects of human exposure to OP sheep dips led to an increase in the number of farmers using SP sheep dips and this in turn heightened concerns about possibilities of further serious incidents of aquatic pollution (Semple *et al.*, 2000; Pearce, 1997).

The increasing concerns about aquatic pollution led to the 1998 Groundwater Regulations under which the Environment Agency (EA) is required to authorise sheep dip disposal sites in order to ensure there are no risks to groundwater. Further, the disposal of spent sheep dip must comply with the Ground Water Regulations (1998) in order to minimise the risk to the environment and strict guidelines are in place (Health and Safety Executive, 1998). The approved method of dip disposal is to spread spent dip on a suitable area of farmland at a ratio of one part spent dip to three parts water or slurry. However, although allowing protection for watercourses, the approved disposal method provides a possibility of adverse effects on the non-target terrestrial invertebrate populations in disposal areas. There is a great deal of evidence that application of similar pesticides to agricultural land have shown adverse effects on communities of non-target invertebrates (Burn, 1989; Cole and Wilkinson, 1985; Hill, 1985; Moreby *et al.*, 1997; Moreby *et al.*, 2001; Sotherton, 1989; Vickerman and Sunderland, 1977). There are also potential effects for birds that rely on the invertebrates as an important component of their diet, either through a localised reduction in their prey populations at a critical time of year or through secondary poisoning following consumption of exposed vegetation or invertebrates (Bishop *et al.*, 1998; Moreby and Southway, 1998; Sotherton, 1989; Evans, 1990).

In 1998, when the regulations were introduced, the majority of sheep dip was already disposed to land. However, the authorisation process was considered likely to increase the area of land used for dip disposal since soakaways (the other main route for on farm disposal at that stage) were unlikely to be approved. The EA is currently required to consult English Nature (EN) and the Countryside Council for Wales (CCW) where a Natura 2000 site i.e., Special Area of Conservation (SAC) for certain habitats and species or a Special Protection Area for birds (SPA) might be affected by an authorisation for disposal.

This thesis examines the impacts on terrestrial invertebrates of sheep dip disposal onto land and assesses the possible consequences for upland breeding birds of reduction in invertebrate food supply. The birds considered are primarily the upland wading species Curlew (*Numenius arquata*), Lapwing (*Vanellus vanellus*), Golden Plover (*Pluvialis apricaria*), Redshank (*Tringa totanus*) and Snipe (*Galinago gallinago*), for which SPAs are often designated and which nest and/or feed in current and potential dip disposal areas. The information is intended to provide further guidance for EA, EN and CCW staff assessing applications for dip disposal near or within Sites of Special Scientific Interest (SSSIs) and Natura 2000 sites.

This study is divided into four main components:

i. A survey of farm practice and compliance with regulatory requirements. This was intended to provide a broad assessment of the nature of the disposal operation to land, including compliance with guidelines, and to allow a preliminary selection of potential areas for further study.
ii. An invertebrate survey of disposal sites and matched control areas of farms where sheep dip disposal to land had been undertaken (referred to as 'historic' farm sites), to investigate whether historic disposal had measurable impacts.
iii. Multifactorial experimental plot investigations of the effects of organophosphates and synthetic pyrethroid dip disposal on invertebrates of upland grassland to test

the impacts of dip disposal under controlled conditions and provide results to compliment those from the historic disposal sites.

iv. The findings are drawn together to provide a greater understanding of the effects of the current dip disposal practice on non-target organisms and possible future uses of this information are suggested.

The potential risks of secondary poisoning of birds were not examined experimentally within this project.

2. ASSESSMENT OF CURRENT DIP DISPOSAL PRACTICE

2.1 Introduction

Farming practices differ across Britain as a result of many factors including terrain, rainfall, quality of land and more sociological factors connected with long held traditions. In accordance with the Groundwater Regulations of 1998 spent sheep dip must be disposed onto suitable farmland that has been approved by the Environment Agency, i.e. relatively flat land at least 10m away from water courses and 50m away from springs, wells or boreholes, with no public access. The spent dip must be applied at a rate of no more than 5000 litres per hectare having first been diluted with three parts water and then spread using suitable equipment such as a slurry tanker (Health and Safety Executive, 1998). These guidelines were intended to protect watercourses and aquatic wildlife, which had suffered significant incidences of pollution resulting from previous dip disposal methods (Pearce, 1997; Virtue and Clayton, 1997). However, the guidelines were not designed to take into account any potential threat to terrestrial wildlife.

A preliminary questionnaire was set up in 1999, during the first year of the implementation of the changes in disposal practice. The questionnaire was intended to determine the range of current dip disposal practices, including the nature and scale of the operation and the type of farmland chosen for disposal. It was also intended to investigate the extent to which farmers in England and Wales complied with the Environment Agency guidelines and, if they deviated, whether the deviations were likely to cause a greater threat to wildlife. This information was intended to aid the assessment of the scale of any environmental risk to farmland, specifically regarding terrestrial organisms. The consequences of any changes in invertebrate prey availability for bird populations could then be investigated. This preliminary assessment of potential effects on the terrestrial environment of the study regions was intended to encompass important details to aid the assessment of likely impact on nature conservation interests. Information from the questionnaire was also intended to establish what were 'typical' dip disposal locations and allow a preliminary selection of potential areas from these for invertebrate sampling.

The questionnaire was intended to provide both qualitative and quantitative information using simple, unambiguous questions to obtain the most accurate responses and avoid confusion and communication failure (S.C.P.R, 1975) and was conducted confidentially to maximise free speech from the respondents by removing the possibility of future identification.

2.2 Methods

Selection of Region

The regions originally considered from which to choose sites for the questionnaires were northern England and Wales. Hill sheep farming is particularly prevalent in these regions and they also contain many important SSSIs. Any detrimental effects of the dip disposal practice could therefore potentially affect these environmentally sensitive areas.

Sorting of Applications

The applications submitted to the EA by farmers for authorisation to dispose of dip in summer 1999 were used to identify sites on SSSIs or other areas of designated conservation value. Applications were sorted at the Newcastle, Cumbria and York

Environment Agency Offices, which had applications for regions where hill sheep farming is prevalent and were easily accessible. Unfortunately it was not possible to visit corresponding Welsh offices for this purpose and farmers taking part in Wales were selected because they were known to the researcher as users of sheep dip.

Identification of Suitable Disposal Sites

In the uplands of northern England a number of potential sites within the Teesdale area were successfully identified, where many farms are comprised almost entirely of SSSI land. Details of more than twenty potential survey sites were obtained from Teesdale, an area of particular interest because of ongoing research and data available about resident and breeding bird species. Details of suitable farms in Cumbria, Northumberland and Wales were more difficult to obtain since the authorisation process was less advanced in these areas. Many farms in these areas also incorporated land that is not within a SSSI and chose to apply for disposal on the land considered to be of lesser conservation value. The additional region of West Yorkshire was chosen because there were insufficient suitable survey sites in the initial search. The number of sites selected in this area were also augmented by farms known previously by the researcher. Therefore, the sites chosen were in four regions including Cumbria, NorthYorkshire/Durham/Northumberland, Wales and West Yorkshire.

Appropriate farms for more detailed assessment of invertebrate fauna and vegetation characteristics were chosen on the basis of information gathered in the questionnaire, as described in Chapter 3. Sites chosen were within or adjacent to SSSIs or other areas of nature conservation importance, and were in Wales, Teesdale and West Yorkshire. A 'worst case' site was also chosen near Derwent Reservoir where there have been repeated dip disposals from many farms in the vicinity. Two sites from each of the three different regions and the 'worst case' site meant that seven different sampling sites were finally chosen in 1999, some of which were replaced with other farms in the same area in 2000 for continuation of the study (see Chapter 3).

Applications to farmers to take part

The questionnaire was carried out on as many sites as possible. A relatively small number of applications for disposal onto environmentally sensitive land were found, resulting in the initial selection of suitable sites for the questionnaire. This number was cut down again by the reluctance of up to 50% of farmers to take part, due to fears of potential adverse consequences resulting from their involvement in the study. Forty-two questionnaires were ultimately completed, including 10 in Teesdale, 6 in Cumbria, 6 in Wales and 20 in West Yorkshire. However, those involved in the questionnaire volunteered additional detail and reasoning behind their responses, some of which is incorporated into the discussion (Section 2.4).

Design of the Questionnaire

The following section details the confidential questionnaire, with comment on the reasons for asking each question.

Questions 1 to 3 were used to identify what chemicals would potentially be put on the land. Questions 4 to 10 were intended gain information about the scale and nature of the dipping and dip disposal process and whether EA guidelines about disposal (Health and Safety Executive, 1998) were being adhered to.

Questions 11a,b and 12 were to establish the quality and basic properties of the land chosen for disposal. The land use is important as it affects the types of invertebrates present and the life-cycle stages during which wading birds are most likely to use the land e.g. for either nesting or feeding.

Questions 13 to 15 were intended to provide general background information and questions 16 and 17 were used to establish on which sites it would be possible to carry out further investigations.

Questions 18 to 20 for farmers no longer using dip were intended to establish what form of alternative protection farmers were using against insect pests, if any, and the reason for this decision. If the alternative plan was not intended to be permanent the previous dipping regime may be returned to, so any potential future disposal could also be worked into risk assessment.

Confidential Farm Questionnaire

1) Do you use sheep dip? (if no go to 18, if yes go to 2)

It was necessary to ascertain whether the farmers still intended to use dip since up to nine months had elapsed between applications to the EA for permission to dispose of spent dip in autumn of 1999, when this questionnaire was carried out. During this time there were national concerns about the safety of handling organophosphate based dips and this may have affected the farmers decisions (MAFF, 1999).

2) What is the name of the dip?

(Active ingredients or product names, from which the active ingredient could be deduced if the farmer was uncertain, were acceptable)

Again, the choice of organophosphate or synthetic pyrethroids active ingredients may have been affected by the concerns about organophosphate based dips.

3) Is there any subsequent treatment; e.g. decontaminant used?

Decontaminant use might change the toxicity of the dip on application to land.

4) How many sheep are dipped?

5) Where is the dip disposed of ?

(e.g. on your own land, to a neighbour's land, mobile dip)

6) What is the method of application to land?

7a) What area is used for disposal e.g.

- **i. acreage**
- **ii. part/whole field**
- **iii. enclosed/open**

7b) Why was the area chosen and how long has it been used for this purpose?

8) What is the quantity /dilution of the dip disposed of?

9) How often and what time of year is dip disposed of?

The timing of dip disposal has implications for terrestrial invertebrates and the birds that feed on them e.g. if disposal occurs when birds are nesting, breeding success may be impaired if invertebrate prey populations are adversely affected by dip disposal. It was therefore important to establish common practice so that disposal timing could be incorporated into risk assessment at a later stage.

10) Is the dip mixed with slurry?

Mixing dip with slurry is permitted in EA guidelines (Health and Safety Executive, 1998) but added another variable when selecting sites for invertebrate sampling.

11a) What type of vegetation is on the disposal area?

(e.g. improved pasture, rough grazing, hay meadow, other)

11b) What is the soil type of the disposal area?

12) Are there clumps of rushes on the disposal area?

The presence of rushes indicates high water content of the soil, likely to result in dip remaining close to the soil surface and hindering its adsorption. Rushes also feature in habitat selection by some nesting birds since they provide cover and indicate soft ground suitable for feeding. Surface contamination by dip on such areas could have particularly adverse affects. This question was designed to give a more accurate indication than asking a direct question about waterlogging, which could involve bias or confusion if respondents had different ideas of what counted as waterlogging (S.C.P.R, 1975).

13) Is the disposal area likely to have any wildlife value?

(e.g. do waders nest or feed on the land?)

If the farmer had observed waders breeding or feeding on the disposal area it could immediately be identified as useful for further study.

14) Do you use other chemical controls on the land i.e. insecticides for leatherjackets?

The application of pesticides other than spent dip added another variable and made such sites unsuitable for invertebrate sampling in the present study.

15) What guidance have you received about dip disposal and was it practical?

Successful compliance with dip disposal guidelines depends on availability of the information, adequate comprehension and ease of implementation. Implementation is more difficult in some circumstances depending on the disposal equipment and land available to the farmer.

16) Would you be happy for me to come back and survey the vegetation and soil type on the disposal area?

17) Could I sample for invertebrates? This would involve taking 24 spadefulls of soil from the disposal area and from an adjacent field (to act as a control) in October and again next spring.

Questions 16 and 17 were intended to establish possible sites for further sampling.

Questions 18 to 20 are for farmers no longer using sheep dip.

18) What alternative to sheep dip do you use to treat the sheep for pests and why?

19) Did you dip regularly before the current legislation came into force?

20) How did you dispose of the dip?

2.3 Results of the Questionnaire

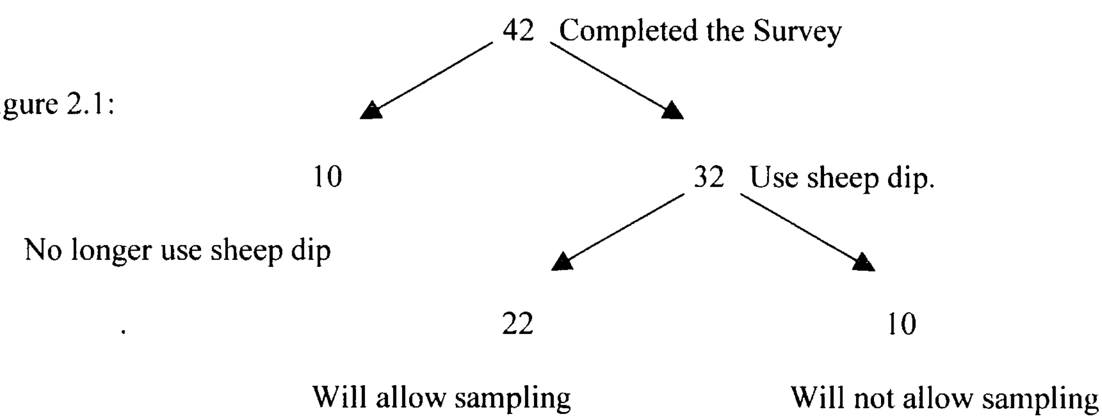
Responses to the Confidential Questionnaire:

Question 1 – Do you use sheep dip?

The 22 sites for which there were dip disposal applications in 1999 all used dip in that year. Of the additional 20 farms surveyed in West Yorkshire, 10 no longer use sheep dip.

Figure 2.1 shows a breakdown of how many farmers were using dip and how many were prepared to allow further sampling:

Figure 2.1:



Farmers using sheep dip then proceeded to question 2, whilst those that did not were asked a different series of questions from the end of the survey sheet. These are discussed in questions 18-20.

Question 2 – What is the name of the dip?

Of the 32 farmers that used dip, 8 were using SPs, whilst 24 were using OPs.

Question 3 – Is there any subsequent treatment, e.g. decontaminant used.

Three of the eight farmers using SP dip were also using a decontaminant, either added to the dip or soil, which imposes an extra cost above that of the actual dip. No farmers using OP dips were also using a decontaminant.

Question 4 – How many sheep are dipped? (Numbers are per annum and are approximate, not taking into account unusual years where conditions require sheep to be dipped twice)

Answers varied between 300 and 6000 but were most commonly between 2000-4000.

Question 5 – Where is the dip disposed of? E.g. own land, neighbours land, mobile dip?

All those questioned disposed of the dip on their own land, although managers of Cumbrian estates commented that in their case they would apply for a single site for all their farms to use.

Question 6 - What is the method of application to the land?

Five out of the 32 dip users used a hosepipe connected to the dip bath or allowed the dip to soak away. The remaining 27 farmers all disposed of the dip via slurry tanker in an approved manner.

Question 7a – What area is used for disposal, e.g. acreage, part or whole fields and enclosed or open land.

Acreage obviously depended on the amount of dip being disposed of but 28 of the 32 farmers disposed on whole, enclosed fields of between 0.5 and 6 ha in area, spreading dip sparingly over as much of the disposal area as possible. One of the 28 disposed of the dip using a long hosepipe, so only covered a small area within the disposal site. The remaining 4, who had used hosepipes or allowed the dip to drain away, had applied for disposal areas similar to those of the other 28.

Question 7b – Why was the area chosen and how long has it been used for this purpose?

The areas were chosen mainly because there were no watercourses or open drains that might become polluted. Convenience and accessibility with a slurry tanker were also important issues. On larger farms the areas had usually been used for disposal before, sometimes for more than 10 years because they had volumes of dip that were too large to let soak away and they had historically been using the current approved method of dip disposal. Smaller farms were usually new to the approved disposal methods, previously having let dip soak away. Disposal areas were therefore generally new in these cases.

Question 8 – What is the volume/ dilution of the dip disposed of?

Dilution ranged between 2:1 and 6:1 volume water to dip, although six farmers out of the 27 spreading with a slurry tanker were mixing it with dry muck rather than diluting with water. Volume disposed of was difficult to assess as the majority of the farmers put an approximately correct but unmeasured amount of dip into a slurry tanker then filled it to the top with water. This led to the variation in dilution rates and volumes dependant on the size of slurry tanker used. Slurry tankers ranged in size from approximately 1500 to 9000 litres capacity. This information was particularly approximate if the slurry tanker had to be hired in for the disposal process and quantities were estimations.

Question 9 – How often and at what time of the year is dip disposed of?

Timing of dipping depends on the other duties of the farmer, outbreaks of scab and the weather, since some conditions are more conducive to scab outbreaks. All the farmers dip between July and October. Those in Teesdale also dip earlier in the year as well, as do some in West Yorkshire and Cumbria depending on the year. Twelve of the 32 farms dipped twice in 1999.

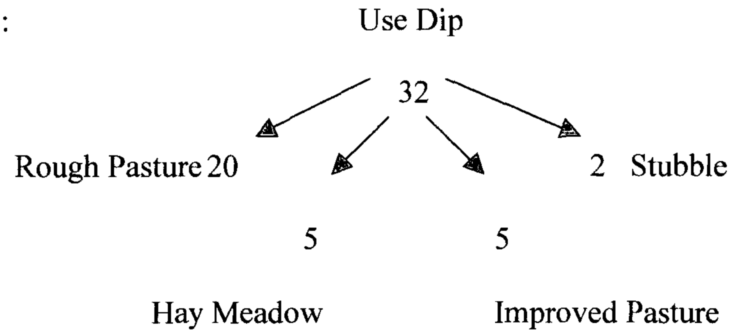
Question 10 – Is the dip mixed with slurry?

None of the farmers claimed to be mixing the dip with slurry, although six were mixing it with dry muck.

Question 11a - What type of vegetation is on the disposal area e.g. improved pasture, rough grazing, hay meadow, other?

20 of the farmers were disposing on rough pasture, five on hay meadow and five on improved pasture. In Cumbria, the farms surveyed included a more diverse range of land quality, partly because of the tendency for larger farm sizes and groups of estate owned tenant farms, allowing for more choice of disposal site, usually using the poorest land possible for this purpose. Two of the farmers in Cumbria dispose on fields of stubble soon after the crop has been removed. Figure 2.2 shows the vegetation on the different types of disposal areas.

Figure 2.2:



Question 11b – What is the soil type of the area? Can I take a sample?

The soil type in Cumbria was mostly believed to be sandy loam, whereas soils in other areas tended to have a high peat content. Due to concern expressed by some farmers, few samples were taken at the time of this first survey.

Question 12 – Are there clumps of rushes on the disposal area?

20 of the farms had rushes present. These mostly corresponded with those farms where disposal was on rough grazing land.

Question 13 – Is the disposal area likely to have any wildlife value, e.g. do waders nest or feed on the land?

15 of the farmers believed their disposal sites might be of wildlife value, particularly in Teesdale. Five others thought nearby land might be important for wildlife but not specifically their disposal sites.

Question 14 – Do you use other chemical controls on the land i.e. insecticides for leatherjackets?

Two of the Cumbrian farms were using insecticides on their cereal crops early in the season and disposing of dip onto stubble. Six others used spot herbicide control, spraying directly onto the individual weeds on improved pasture. The remaining 24 farms used no other chemicals on the land (other than muck as fertiliser).

Question 15 – What guidance have you received about dip disposal and was it practical?

20 of the farmers felt they had received insufficient guidance. Eight believed they had received impractical guidance, since new disposal methods were difficult to comply with and involved extra expense. Four of the 32 farmers that used dip believed they had received adequate and helpful guidance.

Question 16 – Would you be happy for me to come back and survey the vegetation and soil type on the disposal area?

28 of the farmers were happy to help in this way

Question 17 – Could I sample for invertebrates? This would involve taking 24 spadefuls of soil from the disposal area and a suitable uncontaminated control site nearby in October and again next spring.

22 of the farmers were happy to help in this way. The remaining 10 were worried about damage to land, disruption of wildlife and inconvenience.

Questions 18-20 were for farmers that have ceased to use sheep dip:

18) What alternative to sheep dip do you use to treat the sheep for pests and why?

Eight out of the ten farmers who had ceased dipping had changed to injectables. Two had decided to do nothing at all unless symptoms arose. Reasons for these changes were mostly to do with problems of dip disposal. One farmer had been refused permission to dispose of his dip because the disposal site was too close to a group of houses. Others did not have the correct equipment for disposing of the dip, having previously let it soak away and they considered it too expensive to pay for removal or disposal.

19) Did you use dip regularly before the current legislation came into force?

All the farmers questioned had previously used sheep dip.

20) How did you dispose of the dip?

Two of the farmers had spread the dip with muck out of a muck spreader, whilst the other eight had let it soak away.

2.4 Discussion of Questionnaire Results

The questionnaire was intended to assess the range of dip disposal practices and to identify the types of habitat and the extent of the area potentially at risk. Due to the small sample size it is representative of a tiny proportion of farms where dip disposal takes place and is not intended to be a definitive survey of practices either within the study areas or more widely. However, the results are probably representative of the majority of dip disposal practices at large as farmers tended to be uninhibited in their responses due to the confidential nature of the questionnaire.

The questionnaire exposed issues with dipping and disposal methods on hill farms where overall profit margins can be low (BFSC, 2003). In order to maximise profit in the current farming conditions in Britain, particularly since the outbreak of Foot and Mouth disease in

2001 which further complicated British hill sheep farming (DEFRA, 2001), farmers need to find solutions to pest problems that are least costly in financial terms. It is also important to minimise damage or stress to land and livestock, and to keep labour costs low.

The questionnaire highlighted many variations in dipping and dip disposal practices and allowed insight into some of the reasons for the decisions. These variations in dipping and disposal practice are fundamental to the interpretation of the invertebrate studies on the historic farm sites (Chapter 3). Since applying for a disposal permit 10 of the farmers had decided against using dip, predominantly because of problems complying with the new disposal guidelines. Of the 32 farmers that used dip, 8 were using SPs, whilst 24 were using OPs despite concerns about the safety of the OP dips. The reason given for the low percentage of SP users was because SPs were believed to be less effective against sheep scab. Two of the OP users had found SPs to be ineffective and considered that they had lost sheep because of it, thereafter reverting to the use of OPs. Many of the farmers expressed concern about the health risks of using OPs but had found no effective alternatives. For farmers who do not share common grazing land SPs provide effective treatment for other pests such as flies (Meat and Livestock Commission, 2000). Sheep scab is less of a problem for them if there is little or no contact with other flocks. Small numbers of sheep that might have jumped out of enclosed ground or been infected by other sheep jumping in can easily be treated individually by injection. However, the 24 OP users all had common grazing rights and had problems with scab outbreaks in numbers that required the use of dip.

The questionnaire exposed the problem that some farmers had not yet implemented the new disposal guidelines. Five out of the 32 dip users used a hosepipe connected to the dip bath, thereby covering only a small area within the disposal site, or allowed the dip to soak away because of extra cost of hiring someone with a slurry tanker (if they did not have access to one) and the extra time involved with alternative disposal methods. All five had applied for disposal areas and aimed to be better equipped the following year, intending to use the authorised disposal methods or not dip at all. The remaining 27 farmers all disposed of the dip via slurry a tanker in an approved manner but some of the disposal sites had not been approved and were later reduced or completely changed. The disposal sites had mainly been chosen to comply with EA guidelines (Health and Safety Executive, 1998) but convenience and accessibility with a slurry tanker were also important issues. Implementing the new disposal guidelines had been easier on larger farms where the areas had usually been used for disposal before because they had volumes of dip that were too large to let soak away and they had historically been using the current approved method of dip disposal. Smaller farms that were new to the approved disposal methods had previously let dip soak away.

Despite assurances of top-up in dip bath to keep concentrations in line with dip manufacturer and MAFF guidelines, anecdotal evidence suggests that guidelines were not always being followed by farmers, with the dip bath half empty at the completion of dipping. Dip would then be diluted by a much greater amount for disposal than is estimated using practice recommendations based on a full dip bath and results could then underestimate the effects of other dip disposal at recommended strengths. Although the Certificate of Competency is required for the purchase of dip, pressure of farm work dictated that the buyer was not necessarily present during the whole of the dipping period. In addition, when questioned formally in the preliminary questionnaire, farmers answered queries about dip concentrations and best practice correctly whilst admitting to encountering logistical difficulties in following the procedures precisely.

The equipment available can restrict those farmers who intend to carry out all the disposal instructions correctly; for example, muck spreaders are often used when slurry tankers are not available. However, muck spreaders dispense dip much more patchily and at very variable rates making the discharge rate of the dilute dip difficult to measure and control. Tankers or muck spreaders containing the spent dip are also often topped up with an unknown quantity of water or muck, resulting in very variable dip dilution rates. Farmers predominantly spread the dip until it has run out rather than when the designated area for that amount of dip has been covered. This led to problems in subsequent sampling as the exact area of disposal within a designated site may not be obvious (see Chapter 3). Other factors such as storage/degradation time of spent dip before disposal, which depend on the farmer's schedule, weather and accessibility of the disposal sites, are also uncontrollable yet important variables. Further, if disposal is carried out by a third party, because the farmer does not have appropriate equipment, the exact disposal site may not be accurately identified.

Hill sheep are required to be hardy and withstand conditions in which lowland sheep could not thrive (NSA, 2003). Their fleeces are of poorer quality, tending to be smaller and courser than lowland sheep (BWMB, 2002) and hill sheep are generally smaller so they are also less efficient in terms of meat production (NSA, 2003). Even in good years margins are dependent on maintaining low input regimes. The volatile state of the livestock markets, with some sheep being sold for very small sums, has focussed farmers' attentions on ways of reducing overheads. An average OP dip costs between £0.30 and £0.40 per sheep per dipping occasion (Young's, 1999). With the added costs of the permits and licences required to use dip, along with the hiring of slurry tankers and drivers often contracted in for dip disposal, and costs of other handling equipment to deal with the dip, this can amount to more than the individual sheep is worth. These extra expenses, including the disposal permit, are blamed for the reluctance of some farmers to dip sheep or, if they do use dip, for unauthorised disposal methods.

This preliminary survey suggests an inconsistent approach to scab and pest control. Irregular dipping patterns can result in outbreaks of sheep scab that can necessitate farmers that use common grazing land having to dip more than once in the same year. The questionnaire revealed that repeated dipping had occurred on more than a third of the farms using dip in 1999. These farms were predominantly in Teesdale, where co-ordinated dipping times were used in an attempt to eradicate scab off the fell altogether. This can lead to the repeated use of disposal sites on adjacent farms between April and November. However, since not all farmers dispose of the spent dip at the same time (storing it until disposal is possible) dip can be spread over many sites within the area, over several months. The dip will, however, degrade steadily during storage and it is difficult to estimate levels of toxicity in such cases.

The potential for constant presence of sheep dip and the re-inundation of land over the course of the year may present increased environmental hazards to the soil invertebrates and other animals, especially protected bird species that feed on them. Such variation also presents problems in designing experiments, which may represent assessments of 'worst case' scenarios, as well as leading to a multiplicity of scenarios for consideration during risk assessment. The effects of disposing of dip may therefore affect both invertebrates and their dependent predators at many points in their life and breeding cycles. Farmers questioned understood that there could be such environmental issues and were concerned about finding the optimum techniques to avoid affecting the quality and environmental importance of grazing land during the dip disposal process.

3. INVERTEBRATE SAMPLING ON FARM SITES 1999-2000

3.1 Introduction

This part of the investigation was designed to test the hypothesis that, where a site had been used historically for sheep dip disposal, invertebrate densities would be lower and community composition was likely to differ from a comparable 'control' site. As the aim of the study as a whole was to assess the consequences of sheep dip application on land of conservation importance, it was necessary to evaluate the effects of disposal in upland farms within, or close to, conservation areas. Site selection, in northern England and Wales, was over a wide geographical area and reflected the requirement that resultant information would be used for guidance by both English Nature and Countryside Council for Wales staff, as well as the Environment Agency. Evaluation of the effects of dip disposal on terrestrial invertebrates was based on a comparison between historic disposal areas and adjacent uncontaminated control areas, under the same management regime.

Areas used for dip disposal were compared with adjacent uncontaminated areas between October 1999 and June 2000, using four quantitative sampling techniques:

1. Soil samples were taken to determine the densities of soil inhabiting invertebrates
2. Pitfall traps were used to capture surface-active invertebrates
3. Timed suction sampling was used to determine the densities of surface dwelling invertebrates
4. Bird counts

Soil and suction samples were intended to provide invertebrate density comparisons, while pitfall catches were used to give comparative abundance estimates. Pitfall traps can not be used in density measurements as they capture invertebrates from an unknown area (Southwood and Henderson, 2000).

Pitfall catches and suction samples reflect the food available for wader chicks and adults such as golden plover and nesting lapwing, which take surface-active arthropods (Baines 1990, Whittingham *et al.*, 2001). Soil samples tend to include a greater proportion of sedentary invertebrates, such as larval invertebrate life stages, and these are important to soil probing waders such as curlew and oystercatchers (Zwarts and Blomert, 1996). The invertebrate sampling methods are, therefore, appropriate for examining the effects of dip disposal on the food supply of upland birds while the bird counts provided information on use of dip contaminated land by the foraging birds.

Mobile invertebrates rapidly re-colonise areas when pesticide toxicity decreases and the size of area receiving the pesticide application, such as that in dip disposal, influences the recovery rate (Jepson, 1989). In the present study, paired comparisons were made between the densities of invertebrates on disposal and control sites at each farm. Major taxa were compared and the densities of sedentary and active invertebrate groups were also assessed separately, thus allowing the effects of dip disposal to be determined in the absence of recolonisation. Soil samples for density measurements were taken at 7 sites in autumn 1999 and 8 sites in spring 2000. The number of samples was determined by the requirement for an adequate sample size for statistical analysis, moderated by the restraints of time and labour. The autumn samples were taken, when possible, within two weeks of dip disposal to measure the immediate effects of pesticide application. The intention was to sample the same sites in the following spring, before the next disposal occasion, to measure longer-term effects.

Within a single taxon different species may also show different capacities for colonisation. Rushton *et al.* (1989) have suggested that, while active ground beetles will probably re-colonise insecticide treated areas rapidly, less active species will not. The less active species are therefore likely to suffer more persistent local population declines if exposed to dip and this will alter the species composition on the disposal area. In the present study, the large numbers of spiders and ground beetles caught in pitfalls allowed such differential effects to be investigated within two taxa of surface-active invertebrates using CANOCO (Ter Braak, 1988). This multivariate analysis relates the distribution of species to environmental variables, including the effects of dip disposal. Differentiation between sedentary and active groups in the soil samples from paired control and disposal areas allows a broad assessment of the effects of dip disposal on density, in the absence of recolonisation. The multivariate approach allows the more subtle effects of differences between species re-colonising ability to be investigated and is presented in Chapter 4.

Grid references are not available for the farm sites as anonimity was guaranteed for those taking part. The hypothesis that repeated disposal of OP and SP sheep dip could have cumulative deleterious effects on invertebrate populations led to the selection of Derwent as a "worst case" site. Farmers with grazing land abutting Derwent Reservoir (54° 52'N 1° 53'W) are not allowed to dispose of dip on their own land because of potential contamination of the ground water. The Derwent site constituted the disposal area for all the farmers in the reservoir catchment, receiving repeated applications of both OP and SP dips before and during the 1999 sampling period of this study. This provided a baseline site, which was known to be heavily contaminated and where invertebrate populations were expected to be adversely affected.

3.2 Methods

3.2.1 Site selection

The results of the questionnaires (Chapter 2), together with site visits to ascertain whether there was an appropriate control area near the disposal site, were used to select six farms for invertebrate sampling in 1999, Fig. 3.1, Table 3.1. Sites were also chosen on the basis that they were in or adjacent to SSSIs. Two farms from each of Wales (Wales 1 and 2), West Yorkshire (Yorkshire 1 and 2) and Teesdale (Teesdale 1 and 2) were chosen which, with the addition of the "worst case" site at Derwent Reservoir, made seven sites in total in 1999. Control sites were chosen from fields adjacent to the disposal field at each farm. The controls had not had dip applied to them and were selected to be, as far as possible, of similar soil type and under the same management regime as the disposal sites.

It was necessary to change some sampling sites in 2000, the reasons for which are detailed in the site descriptions (Section 3.2.2). The same site characteristics were measured for the new sites as in 1999 (Table 3.1). Invertebrate sampling was carried out in spring 2000 on Teesdale 1, 1A and 2, Wales 2 and 3, Derwent, Yorkshire 3 and 4. Pitfall collections were made at Teesdale 1, 1A, 2, 3, 4, 5 and 6 and Yorkshire 3, 4 and 5 from May to November 2000. Bird watching was also carried out in spring 2000 at all the Teesdale sites and Yorkshire 2, 3, 5 and 6.

3.2.2 Site Descriptions

Approximate locations of the sampling areas are shown in Figure 3.1. Derwent was near Derwent Reservoir in Northumberland, south of Corbridge. Wales 1, 2 and 3 were near Rhayader in the Elan Valley and Yorkshire 1, 2, 3, 4, 5 and 6 were near or bordering Oxenhope Moor between Halifax and Keighley in West Yorkshire. Teesdale 1, 1A, 2, 3 and 4 were near Langdon Beck in the Tees Valley and Teesdale 5 and 6 were at the edge of Lune Moor near Middleton-in-Teesdale. Although grid references and photographs of individual sites are not included because of anonymity agreements with the farmers, the following site descriptions are intended to explain sampling decisions and aid repeatability of the investigation. Vegetation on the sites was identified according to Rose (1981).

3.2.2.1 Derwent

Worst case scenario

Sampled September 1999 and May 2000

Sampling methods: soil sampling with heat extraction 1999 and 2000, earthworm sampling in 1999.

At Derwent a field, measuring approximately 0.5 ha, was used for disposal, leaving up to a 2m boundary around the field edges, with areas of approximately 10 x 20 m, beyond the turning circles and area of distribution of the tankers, at the corners. The control area for this site was in part of the same field as the disposal site, since there was not another comparable ungrazed field nearby. Corners of the field that were beyond the reach of the heavy farm vehicles (because of large turning circles) were utilised as the control (untreated area), keeping as far away from the walls as possible so as to lessen any possible edge effects. The vegetation was predominantly *Agrostis* and *Festuca* grasses interspersed with thistles and other grassland weeds. The field was previously used for grazing and was bordered to the North and East with pasture, separated by dry stone walls, and to the South and West by wooded areas.

Derwent was selected to represent a probable worst case scenario site. The farmers in the catchment area of the reservoir were not permitted to dispose of sheep dip onto their land as it might cause pollution of the water. They therefore disposed, using their own equipment, onto one field set aside by Northumbrian Water for sheep dip disposal. This field received multiple disposals of both SP and OP dips during the April-November dipping season. The exact amount of dip disposed and the proportions of each dip type were not known.

3.2.2.2 Wales 1
Sampled September 1999

Sampling methods: soil sampling with heat extraction

The area designated for disposal was over 1 ha at Wales 1 and consisted of very rough grazing land with *Nardus* spp. and *Sphagnum* mosses overlying peaty soil with the highest organic content of any of the historic sites (86.5%, Table 3.1). The disposal area was used for the first time in spring 1999 to dispose of OP dip. Later it was not certain that the actual disposal site had been sampled since the information from the farmer on the actual area used was not thought to be reliable. Some of the soil samples may therefore have been taken from outside the actual disposal area. In view of this uncertainty this site was not re-sampled in 2000.

3.2.2.3 Wales 2

Sampled November 1999 and May 2000

Sampling methods: soil sampling with heat extraction

At Wales 2 the disposal area and control were part of the same field, which was over 1 ha in area. The field was used as pasture and consisted mainly of seeded rye grasses. SP with decontaminant was applied at the end of October in 1999. This application followed at least 5 years of use of OP with a similar disposal method. Although the exact boundary of the disposal area was known, disposal was done by bucketing out the dip rather than using a slurry tanker. The dip therefore probably fell on localised patches of land, within the general disposal area.

3.2.2.4 Wales 3

Sampled May 2000

Sampling methods: soil sampling with heat extraction

The disposal area at Wales 3 was on a large field of 2-3 ha of improved pasture consisting of predominantly of rye grasses. A matching adjacent field was used for the control. SP was disposed in autumn 1999 on an area that had not previously received dip. Wales 3 was introduced in 2000 to replace Wales 1 because of the uncertainties about the disposal location in the previous site.

3.2.2.5 Yorkshire 1

Sampled October 1999

Sampling methods: soil sampling with heat extraction

The disposal site Yorkshire 1 measured approximately 0.25 ha and was on improved pasture with seeded rye grasses bordered by dry stone walls, quite close to the farm buildings and also to open moorland. A matching adjacent field of comparable size was used as the control. SP with decontaminant was applied to the disposal area in October 1999. The disposal site had not been used before. This site could not be utilised in 2000 since it was not made available.

3.2.2.6 Yorkshire 2

Sampled November 1999:

Sampling methods: soil sampling with heat extraction, earthworm counts

April - June 2000: bird counts

At Yorkshire 2 the disposal and matching control areas were adjacent fields that measured approximately 0.5 ha. The fields were used as hay meadow and contained mainly *Poa* grasses and clover. They were used for one or two crops of hay per year and grazed in autumn and winter by sheep and cattle. The SP was applied to the disposal area in October 1999. Above both fields there is a small road for access to other farms with small wooded areas beyond. Moorland adjoining fields across the road is visible from the disposal site. Due to the use of the fields as hay meadows they could not be utilised for pitfall traps or soil sampling in Spring 2000, but it was possible to count birds from the road, thereby not disturbing the crop.

3.2.2.7 Yorkshire 3

Sampled April - June 2000

Sampling methods: soil sampling with heat extraction, pitfall traps, suction sampling, bird counts

At Yorkshire 3 the control and disposal areas each covered approximately 0.25 ha of land within a total area of 1 ha and were matching adjacent expanses of improved pasture which merged into rougher grazing further up the hillside and then into moorland. The vegetation cover consisted of *Nardus stricta* and *Festuca* grasses on uneven ground with large tussocks at irregular intervals and some *Juncus*. The ground was waterlogged in places and *Sphagnum* mosses were prevalent. SP was applied to the disposal area in autumn 1999. Yorkshire 3 was a replacement for Site Yorkshire 1 in 2000.

3.2.2.8 Yorkshire 4

Sampled May - June 2000

Sampling methods: soil sampling with heat extraction, pitfall traps, suction samples

The matching disposal and control areas used at Yorkshire 4 in 2000 each cover an area of around 0.15 ha and were on improved pasture, with *Poa* grasses, used mostly for grazing. SP dip was applied to the disposal area in autumn 1999. OP based dips had been used and disposed of on the disposal site until the last few years (the farmer was not sure of the actual year of the changeover) when SPs had been used instead. The site was close to the farmhouse and heavily grazed at lambing time, which made it unsuitable for bird counts. A B-road runs along the top of both the control and adjacent disposal fields. Yorkshire 4 was particularly useful for this study since it had been used as the dip disposal site for more than 30 years and replaced Yorkshire 2 in 2000. However, permission was not granted for disposal to continue and an alternative disposal area was used in 2001.

3.2.2.9 Yorkshire 5
Sampled April - June 2000

Sampling methods: pitfall traps, suction samples, bird counts

Yorkshire 5 had matching disposal and control areas that measured approximately 0.5 ha and the estimated disposal coverage was 0.25 ha during any one disposal occasion. The disposal and control areas consisted of improved pasture in two adjacent fields enclosed by dry stone walls and bordering on open moorland on two sides. The vegetation cover was predominantly short cropped *Agrostis* grasses and the fields were used for grazing by sheep for most of the year. OP was applied to the disposal area in autumn 1999.

3.2.2.10 Yorkshire 6
Sampled April - June 2000

Sampling methods: bird counts

Yorkshire 6 had comparable control and disposal areas each measuring approximately 0.5 ha in adjacent fields. The fields contained improved pasture with *Agrostis* grasses that was grazed by sheep for most of the year and were bounded by dry stone walls with footpaths running along the top and bottom of both fields. OP dip was applied to the disposal area in Autumn 1999. Pitfall traps were not laid at this site because of disturbance to the sheep at lambing time.

3.2.2.11 Teesdale 1
Sampled November 1999

Sampling methods: soil sampling with heat extraction, earthworm counts.

April - June 2000

Sampling methods: soil sampling with heat extraction, pitfall traps, suction samples, bird counts

In 1999 Teesdale 1 had a disposal site and an adjacent matching control area that measured approximately 0.10 ha and were within the same field. The field was used as improved pasture for sheep grazing and as hay meadow for one crop per year. Vegetation cover was mainly rough meadow grass *Poa trivialis* with some *Juncus* in waterlogged patches. Along the top of the control and disposal areas at Teesdale 1 was a minor road leading to other farms. The site was surrounded by fields on a valley side with moorland above and a river running along the valley bottom. The site had been used for dip disposal for up to 5 years prior to 1999, sometimes with more than one dip application per year. In 1999 OP was applied to the disposal area in November. This site was not permitted for use for dip disposal in 2000 so the new site Teesdale 1A on the same farm was also sampled in Spring/Summer 2000.

3.2.2.12 Teesdale 1A
Sampled April - June 2000

Sampling methods; soil sampling with heat extraction, pitfall traps, suction samples, bird counts

N.B. This was the only site where sampling both pre and post dip disposal was possible.

Teesdale 1A had a possible disposal area of approximately 0.10 ha. A matching control site was used in an adjacent field. The Teesdale 1A disposal area was close to the previously used Teesdale 1 and shared similar characteristics, also being improved pasture rough meadow grass *Poa trivialis*, used for one hay crop per year. However, Teesdale 1A had not been used for dip disposal previously. The new site was further from the road and closer to the river and valley bottom, therefore on a much shallower slope (Table 3.1). Soil sampling was carried out before and after the spring disposal of some OP dip that had been stored from dipping the previous year. The dip might have slightly degraded chemically during storage, particularly as disposal was after the last date recommended for use by the manufacturer. Teesdale 1A was the replacement disposal site for Teesdale 1 in 2000.

3.2.2.13 Teesdale 2

Sampled November 1999 and April - June 2000

Sampling methods: soil sampling with heat extraction, pitfall traps in 2000, suction samples in 2000, bird counts in 2000, earthworm sampling in 1999.

The disposal and control areas on Teesdale 2 measured approximately 0.5 ha and were in matching adjacent fields used for grazing by sheep and cattle and as hay meadow. Both fields predominantly contained rough meadow grass *Poa trivialis*. They were close to the river in the valley bottom and bordered above by rougher grazing which changes into moorland further up the valley side. The control site used in 1999 was found to have a lower organic content (12.2%) than the disposal area (19.4%), so it was abandoned. This difference in organic content was greater than any of the other sites. The control used in 2000 was more comparable to the disposal area in pH values and organic content (Table 3.1). The possible disposal area encompassed two large fields, each of about 1 ha in size. In autumn 1999 OP dip was spread in the first field until the tanker was empty. The actual disposal area used was difficult to gauge and some of the sampling in both years may have taken place outside the area. The large size of the possible disposal area meant that disposing on exactly the same area in successive years could be avoided. In practice, the farmer tried not to make multiple applications on the same area. This increased the problem of identifying the exact disposal location. Neither field had been used for disposal prior to 1999.

3.2.2.14 Teesdale 3

Sampled April - June 2000

Sampling methods: pitfall traps, suction samples, bird counts

The adjacent disposal and control sites at Teesdale 3 each measured approximately 0.10 ha. The land was used for grazing and as hay meadow and contains rough meadow grass *Poa trivialis*. OP dip was applied to the disposal site in autumn 1999. A river ran along the bottom of the fields and a minor road passed along the top. To the left of the control field was a barn, which was used to store animal feed.

3.2.2.15 Teesdale 4

Sampled April - June 2000

Sampling methods: pitfall traps, suction samples, bird counts

Teesdale 4 had a dip disposal site measuring approximately 0.25 ha adjacent to an area renowned for the presence of a black grouse lek. The disposal and control sites were comparable fields of rough grazing land containing *Festuca* and *Agrostis* grasses and *Juncus* in waterlogged areas. The land was close to a relatively busy link road across the moorland. Disposal of OP dip had occurred the previous autumn.

3.2.2.16 Teesdale 5

Sampled 2000

Sampling methods: pitfall traps, suction samples, bird counts

The disposal and control areas at Teesdale 5 each measured approximately 0.1 ha and were part of one large field divided by a seldom-used access track. The field contained rough grazing land that merged into moorland to one side of the control area and contained *Nardus* spp. and *Agrostis* grasses with some *Juncus* interspersed. The disposal area had a B-road running down one side of it, often utilised by heavy vehicles from nearby quarries. Spent OP dip was spread onto the land by driving along the access track, expelling the diluted dip out of the side of a slurry tanker until it was empty. This resulted in a long strip of land becoming the potential disposal area. The actual area covered was unclear. Choosing appropriate locations for sampling was therefore quite difficult and judgement on the area to be covered by the bird counts fairly arbitrary. Disposal had occurred the previous autumn.

3.2.2.17 Teesdale 6

Sampled April - June 2000

Sampling methods: pitfall traps, suction samples, bird counts

Teesdale 6 had unmarked control and disposal areas measuring approximately 0.1 ha, which consisted of rough grazing land with *Nardus stricta* and *Festuca* grasses and some *Juncus* merging into moorland further away from the farm. The land was at the top of a river-cut valley and both areas contained deep drainage ditches to prevent waterlogging of the peaty soil. Dip spreading of was on an area accessible to heavy farm vehicles, namely on a plateau at the hilltop. The actual area covered was uncertain and may have been missed by some of the sampling. Disposal of OP dip had occurred the previous autumn.

3.2.3 Site characteristics

At each site, the timing of dip application, altitude and slope were determined, land use described and pH and organic content measured (Table 3.1). Organic content and pH measurements were based on 12 replicate $0.001m^2$ soil cores taken from each disposal and control area. Six cores were used for pH measurement and the other six dried to constant weight before ignition, at 440 °C for 4h. pH was measured by stirring 2g of each soil sample in 20 ml of 0.1M KCl solution and allowing to stand before testing with a pH meter. Organic content was calculated from the loss of weight on ignition. In each case the mean of the six values was calculated (Table 3.1).

3.2.4 Timing of soil sampling

Due to differing farming practices, soil sampling for invertebrates was carried out over three months in 1999, between September and November, depending on when the farmers had disposed of the dip. Where possible, samples were taken approximately two weeks after the dip had been spread. The aim was to allow time for the dip to have an impact on the invertebrates, without significant recovery and recolonisation, and for the dip concentration to drop to a safe level for handling by the observer. Longer-term effects were investigated by resampling, where possible, in spring 2000 before another disposal had been carried out. The time intervals between dip application and soil sampling are shown in Table 3.1.

3.2.5 Invertebrate sampling

Soil Invertebrates

In order to determine densities of soil invertebrates, 12 soil samples were taken and bagged individually, each approximately 17.5 cm^3, from each disposal and each control site at each farm. Random sampling was stratified to cover the area where disposal was understood to have taken place and an equivalent area was sampled on the control site. Soil samples were heat extracted into 70% alcohol using Berlese funnels (Southwood and Henderson, 2000) for one week. Invertebrates were sorted and identified to family level (or as precise a level as possible depending on the stage of development of the individuals).

In 1999 12cm^3 soil samples were taken from both the control and disposal sites at Yorkshire 2, Teesdale 1 and 2 and Derwent investigate earthworm densities. The samples were sorted by hand on site or on return to the laboratory.

Surface-active invertebrates

The sites added in 2000, particularly those that were not sampled for soil invertebrates using Berlese extraction, were intended to increase the sample size for the purposes of multivariate analysis. Pitfall sampling was carried out from mid-May to the end of July 2000, 6 to 8 months after the last dip disposal on 10 farm sites, 7 in Teesdale (Teesdale 1-6) and 3 in Yorkshire (Yorkshire 3-5). Six pitfalls, 7 cm in diameter with approximately 50ml of ethylene glycol (Clark and Blom, 1992), were sunk level to the ground surface in a straight line at approximately 2 m intervals in each control and disposal area. Pitfalls were in position throughout the catching period and were collected and replaced at fortnightly intervals. Bugs, carabid beetles and spiders from pitfall catches were identified to species level.

Suction sampling for surface-active invertebrates was carried out for two 30s intervals at each pitfall site, once at each farm, in June. Suction sampling was carried out using an Echo "Blower-vacuum" with an extension sampling tube (aperture 0.01 m^2) (Macleod, *et al.*, 1994). Timing of suction sampling was determined by the weather, as sampling on wet vegetation is not possible.

3.2.6 Invertebrate Identification

The invertebrates collected were identified and sorted into major taxa according to Chinery (1993). Beetles were identified to species level by Dr J. Butterfield (Durham University) and spiders and bugs were identified to species level by Dr J. Woodward.

3.2.7 Bird counts

The purpose of the bird counts was to determine the usage of the habitat type during the breeding season rather than to estimate the relative abundance in treated and untreated areas. Bird counts were carried out at approximately fortnightly intervals between April and June 2000 on 11 farm sites, 7 in Teesdale and 4 in Yorkshire. This entailed early morning visits to each site, walking through the control and disposal areas, or observing from the field boundary where entrance to fields was not possible. Bird species and numbers were identified and recorded for ten minutes on both the control and disposal seen areas on each visit. The sequence of visits was rotated to observe the sites at different times of the day.

Birds were identified with the help of Dr D. Baines (Game Conservancy Trust) and according to Peterson *et al.* (1993)

3.2.8 Statistical analysis

Density comparisons (soil samples and suction samples)

In addition to comparing the total invertebrates from each site invertebrate taxa were analysed separately in the following groups:

i. **Beetles, flies, tipulid larvae** and **earthworms** (important taxa for feeding upland birds)

ii. **Sedentary arthropods** (weevils, beetle larvae, tipulid larvae, all other fly larvae, hemiptera, lepidoptera, sawfly caterpillars) and **active arthropods** (carabids, staphilinids, other beetle adults, flies, ants, other hymenoptera, spiders, harvestmen, centipedes), to distinguish between invertebrate groups with poor and high potential for re-colonising disposal areas on the farm sites.

iii. **Non-arthropods** (earthworms, slugs and snails)

Analyses were carried out on log-transformed data to normalise the distribution (Fowler *et al.*, 1998). This was necessary due to the large variances in the data that arise due to the patchy distribution of natural invertebrate populations (Wallwork, 1976).

Student's t-tests were used to compare invertebrate densities (soil and suction samples) and comparative abundances (pitfall catches) from control and disposal areas at the farm sites and are presented, with geometric mean densities Tables 3.2 to 3.7. At Teesdale 1A a predisposal sample allowed further analysis to determine the percentage change in invertebrate numbers in control and disposal plots pre and post disposal. Chi-square analysis was used to test for goodness of fit (Appendix 1).

Multivariate analysis was carried out on the spiders and carabids identified from the pitfall catches, the method for which is detailed in Chapter 4.

Table 3.1 Site characteristics and dip disposal information for the farm sites in 1999 and 2000

Site	Dip Type	1999 Interval ^	2000 Interval ^	Altitude (m)*	Slope (degrees)	pH	Organic Content %	Landuse
Derwent c.	N/A	N/A	N/A	220	15-20	4.5	7.63	Setaside
Derwent d.	OP/SP	<30 days	6 months	220	15-20	5.2	8.54	Setaside
Teesdale 1 c.	N/A	N/A	N/A	420	20-25	4.9	21.6	Hay Meadow
Teesdale 1 d.	OP	10 days	6 months	420	20-25	4.7	18.8	Hay Meadow
Teesdale 1A c.	N/A	N/A	N/A	410	5-10	4.6	19.4	Hay Meadow
Teesdale 1A d.	OP	N/A	14 days	410	5-10	4.7	20.2	Hay Meadow
Teesdale 2 c.	N/A	N/A	N/A	420	0-5	4.2	18.7+	Hay Meadow
Teesdale 2 d.	OP	14 days	6 months	420	0-5	4.4	19.4	Hay Meadow
Teesdale 3 c.	N/A	N/A	N/A	420	0-5	4.8	18.5	Improved Pasture
Teesdale 3 d.	OP	N/A	6 months	420	0-5	4.9	19	Improved Pasture
Teesdale 4 c.	N/A	N/A	N/A	430	0-5	4.6	19.2	Improved Pasture
Teesdale 4 d.	OP	N/A	6 months	430	0-5	4.7	18.7	Improved Pasture
Teesdale 5 c.	N/A	N/A	N/A	450	0-5	4.5	20	Rough Grazing
Teesdale 5 d.	OP	N/A	6 months	450	0-5	4.5	19.8	Rough Grazing
Teesdale 6 c.	N/A	N/A	N/A	460	0-5	4.3	21.1	Rough Grazing
Teesdale 6 d.	OP	N/A	6 months	460	0-5	4.2	21.2	Rough Grazing
Wales 1 c.	N/A	N/A	N/A	450	unknown	3.9	86.5	Rough Grazing
Wales 1 d.	OP	>60 days	N/A	450	unknown	4.2	unknown	Rough Grazing
Wales 2 c.	N/A	N/A	N/A	400	10-15	3.8	38.2	Improved Pasture
Wales 2 d.	SP(dec)	10 days	6 months	400	10-15	3.9	40.1	Improved Pasture
Wales 3 c.	N/A	N/A	N/A	380	5-10	4.3	40.2	Improved Pasture
Wales 3 d.	SP	N/A	6 months	380	5-10	3.9	46.3	Improved Pasture
Yorkshire 1 c.	N/A	N/A	N/A	420	5-10	4.2	17.8	Improved Pasture
Yorkshire 1 d.	SP(dec)	10 days	N/A	420	5-10	4.5	18.1	Improved Pasture
Yorkshire 2 c.	N/A	N/A	N/A	350	10-15	4.3	18.4	Hay Meadow
Yorkshire 2 d.	SP	21 days	N/A	350	10-15	4.6	18.9	Hay Meadow
Yorkshire 3 c.	N/A	N/A	N/A	350	0-5	3.2	21.4	Rough Grazing
Yorkshire 3 d.	SP	N/A	6 months	350	0-5	3.5	23.1	Rough Grazing
Yorkshire 4 c.	N/A	N/A	N/A	300	5-10	4.3	16.5	Hay Meadow
Yorkshire 4 d.	SP	N/A	6 months	300	5-10	3.8	17.1	Hay Meadow
Yorkshire 5 c.	N/A	N/A	N/A	350	5-10	4.4	18.2	Improved Pasture
Yorkshire 5 d.	OP	N/A	6 months	350	5-10	4.3	17.9	Improved Pasture
Yorkshire 6 c.	N/A	N/A	N/A	400	5-10	N/A	unknown	Improved Pasture
Yorkshire 6 d.	OP	N/A	6 months	400	5-10	N/A	unknown	Improved Pasture

c. = control site
d. = disposal site
^ = interval between dip application and soil sampling
* = m above sea level
N/A = site not used for disposal or not resampled at this time
+ = control used in 2000 (see section 3.2.2.13)

Figure 3.1: Physical Map of Britain Showing Sheep Dip Disposal Sites Investigated 1999 – 2002

3.3 Results

3.3.1 Soil Invertebrates Autumn 1999

Earthworm Densities

There were no significant differences in earthworm densities between the control and disposal areas on any of the sampling sites.

Total Invertebrate Densities

Mean densities of total invertebrates were significantly lower on disposal areas than on control areas at three sites, Yorkshire 1, Teesdale 1 and Derwent, in autumn 1999. Disposal and control areas at Teesdale 2, Wales 1 and 2 and Yorkshire 2 showed no significant differences, although at Teesdale 2 invertebrate densities were 70% higher on the disposal area than on the control (Table 3.2).

Beetles, Flies and Tipulid Larvae

Beetles, flies and tipulid larvae, in particular, are important food for birds (Baines, 1990 Galbraith *et al.*, 1993, Whittingham *et al.*, 2001) and these abundant groups have been analysed separately (Table 3.3). Beetle densities on disposal areas were about half the level on the controls at five out of seven sites and were significantly lower at Yorkshire 1, Teesdale 1 and Derwent. Flies showed a high degree of between sample variation but were at significantly lower densities on the disposal areas at the first two sites but not at Derwent, while lower densities of tipulid larvae on the disposal area were significant at Teesdale 1 only. There were significantly higher numbers of tipulids on the disposal area than on the control at Yorkshire 2 and Teesdale 2 and beetle densities were also significantly higher on the disposal area of Yorkshire 2 (Table 3.3).

Table 3.2: Earthworm densities and geometric mean densities of invertebrates, from log-transformed data, extracted from soil samples from control and disposal areas in 1999

Site	Sampling Date	Dip Type	Approx.interval since dip application		Earthworm Densities Per Site	Invertebrate Geometric mean /m^2	t-tests for total no.s of invertebrates		
							t	df	P
Wales 1	02/09/99	OP	several months	C D	N/A N/A	1228.08 818.29	0.78	18	NS
Wales 2	06/11/99	SP (Dec)	few days	C D	N/A N/A	1014.53 836.90	1.08	22	NS
Yorkshire 1	31/10/99	SP (Dec)	10 days	C D	N/A N/A	2298.78 402.61	2.14	22	<0.05
Yorkshire 2	03/11/99	SP	21 days	C D	41 33	740.24 964.57	-1.25	22	NS
Teesdale 1	12/11/99	OP	10 days	C D	72 62	351.67 139.76	3.4	22	<0.01
Teesdale 2	17/11/99	OP	14 days	C D	11 43	158.04 269.39	-2.01	22	NS
Derwent	02/09/99	OP SP	within month	C D	41 53	2515.59 1092.24	4.17	22	<0.01

C = Control
D = Disposal site

Active and Sedentary Invertebrates

Grouping the invertebrates as active (mainly predators) and sedentary, the densities of predatory arthropods were significantly lower on the disposal areas than on the controls at Wales 2 and Derwent only. However, there were significantly lower densities of sedentary arthropods on the disposal than on the control areas at Yorkshire 1, Teesdale 1 and Derwent but significantly higher densities on the disposal area at Teesdale 2. There were no significant differences between the densities of non-arthropod invertebrates on disposal and control areas (Table 3.4)

Table 3.3: Comparison of geometric mean densities of beetles, flies and tipulid larvae from soil samples within control and sheep dip disposal Sites in 1999.

Site	Date	Dip Type		Beetles				Flies				Tipulid Larvae			
				geometric mean /m^2	t-test data t	df	P	geometric mean /m^2	t-test data t	df	P	geometric mean /m^2	t-test data t	df	P
Wales 1	02/09/99	OP	Control	387.27				288.98				55.84			
			Disposal	192.33	1.52	18	NS	86.53	1.14	18	NS	45.71	0.16	18	NS
Wales 2	06/11/99	SP	Control	337.96				163.92				101.22			
		(Dec)	Disposal	290.29	1.33	22	NS	129.96	0.79	22	NS	54.86	1.25	22	NS
Yorkshire 1	31/10/99	SP	Control	138.45				3205.22				48.98			
		(Dec)	Disposal	81.96	2.22	22	<0.05	154.12	2.15	22	<0.05	48.98	0.14	22	NS
Yorkshire 2	03/11/99	SP	Control	66.29				361.14				32.65			
			Disposal	116.24	-2.15	22	<0.05	835.27	-0.78	22	NS	46.04	-2.26	22	<0.05
Teesdale 1	12/11/99	OP	Control	83.27				148.57				96.33			
			Disposal	32.65	4.8	22	<0.01	46.37	4.48	22	<0.01	40.82	3.14	22	<0.01
Teesdale 2	17/11/99	OP	Control	79.35				46.04				60.73			
			Disposal	155.43	-1.85	22	NS	54.53	-0.67	22	NS	108.41	-3.1	22	<0.01
Derwent	02/09/99	OP	Control	676.24				597.22				54.86			
			Disposal	295.84	2.58	22	<0.05	237.71	1.05	22	NS	37.55	1.13	22	NS

Beetles: carabids, staphilinids, weevils, other beetles, beetle larvae
Flies: flies and fly larvae, excluding tipulid larvae

NB. Student's t-tests were carried out on log-transformed data

Table 3.4: Comparison of geometric mean densities of active arthropods, more sedentary arthropods/ soil dwellers and non-arthropods in soil samples from control and sheep dip disposal sites in 1999.

Site	Date	Dip Type		Active arthropods (mainly predators) geometric mean /m^2	t	df	P	Soil and more sedentary arthropods geometric mean /m^2	t	df	P	Non-arthropods geometric mean /m^2	t	df	P
Wales 1	02/09/99	OP	Control	705.53				480.47				35.92			
			Disposal	664.70	-0.4	18	NS	179.27	1.82	18	NS	41.14	-0.4	18	NS
Wales 2	06/11/99	SP (Dec)	Control	661.47				299.28				88.49			
			Disposal	493.23	2.15	22	<0.05	284.42	0.83	22	NS	99.59	0.16	22	NS
Yorkshire 1	31/10/99	SP (Dec)	Control	113.22				2738.75				93.39			
			Disposal	69.09	2.03	22	NS	217.83	2.4	22	<0.05	137.47	-1.6	22	NS
Yorkshire 2	03/11/99	SP	Control	93.52				634.30				60.41			
			Disposal	125.55	-1.1	22	NS	838.02	-1.3	22	NS	57.80	0.24	22	NS
Teesdale 1	12/11/99	OP	Control	93.15				159.10				59.10			
			Disposal	61.43	1.48	22	NS	57.39	5.34	22	<0.01	51.92	0.2	22	NS
Teesdale 2	17/11/99	OP	Control	84.24				92.47				46.04			
			Disposal	132.29	-1.65	22	NS	158.67	-2.42	22	<0.05	38.20	0.83	22	NS
Derwent	02/09/99	OP	Control	930.02				1454.80				146.94			
			Disposal	466.65	3.49	22	<0.01	572.93	4.4	22	<0.01	96.33	1.83	22	NS

Active Arthropods (mainly predators): carabids, staphilinids, other beetle adults, flies, ants, other hymenoptera, spiders, harvestmen, centipedes
Soil and more sedentary arthropods: weevils, beetle larvae, tipulid larvae, all other fly larvae, hemiptera, lepidoptera and sawfly caterpillars
Non-arthropods: earthworms, slugs and snails.

NB. Sudent's t-tests were carried out on log-transformed data

3.3.1.1 Spring 2000

Total Invertebrate Densities

In spring 2000, mean densities of invertebrates were significantly lower on areas where disposal had taken place in autumn 1999 than on control fields at Derwent, Teesdale 1, Yorkshire 3 and Yorkshire 4 (Table 3.5). At Wales 2, however, the densities of invertebrates were significantly higher on the disposal than on the control area. At Teesdale 1A, where sampling was possible before disposal, densities were significantly higher on the disposal area than on the control area, before the dip had been applied ($t_{22} = -2.15$, $p<0.05$). Fourteen days after disposal, invertebrate densities had risen significantly (14%) on the control area

Table 3.5: Geometric mean densities of invertebrates extracted from soil samples from control and disposal areas in spring 2000

Site	Sampling Date	Dip Type	Approx.interval since dip application		Geometric mean / m^2	t-tests for total numbers of invertebrates		
						t	df	P
Derwent	04/05/00	SP/OP	several months	C D	1835.45 810.22	4.06	22	<0.01
Teesdale 1	18/04/00	OP	several months	C D	562.79 307.06	2.68	22	<0.05
Teesdale 1A (pre-disposal)	16/05/00	OP	pre-disposal	C D	737.48 1193.07	-2.15	22	<0.05
Teesdale 1A (post disposal)	11/07/00	OP	14 days	C D	844.04 926.92	-0.18	22	NS
Teesdale 2	18/04/00	OP	several months	C D	687.22 781.45	0.2	22	NS
Yorkshire 3	18/05/00	SP	several months	C D	1142.00 511.71	3.41	22	<0.01
Yorkshire 4	02/06/00	SP	several months	C D	1175.80 249.62	7	22	<0.01
Wales 2	24/05/00	SP	several months	C D	895.76 1664.79	-2.33	22	<0.05
Wales 3	24/05/00	SP	several months	C D	1027.22 1018.28	-0.9	22	NS

C = Control
D = Disposal site
NB. Student's t-tests were carried out on log-transformed data

comparing the difference in invertebrate densities between pre and post disposal ($\chi^2 = 7.2$, $p<0.05$) and significantly decreased (-22%) on the disposal area ($\chi^2 = 33.4$, $p<0.05$).

Beetles, Flies and Tipulid larvae

The mean densities of beetles on the disposal sites at Derwent, Teesdale 1, Yorkshire 3 and Yorkshire 4 were about half those on the control sites and were significantly lower in each case (Table 3.6). At Teesdale 2, Wales 2 and Wales 3 the beetle densities on the disposal sites did not differ significantly from the control areas. At Teesdale 1A beetle density did not alter significantly on the control site between pre and post disposal sampling but there was a significant decrease (-17%) in beetles on the disposal site ($\chi^2 = 4.1$, $p<0.05$). Flies showed a similar pattern to beetles with significant differences at Derwent, Yorkshire 3 and Yorkshire 4. At Teesdale 1A fly densities increased significantly (69%) on the control area ($\chi^2 = 40.6$, $p<0.05$), and decreased significantly (-44%) on the disposal area ($\chi^2 = 95.1$, $p<0.05$) between pre and post dip disposal sampling. Yorkshire 4 was the only site where tipulid densities differed significantly on control and disposal areas, with densities four times higher on the control areas.

Active and Sedentary Invertebrates

When active and sedentary arthropods were compared, both groups were at significantly lower densities on the disposal areas at Derwent, Yorkshire 3 and Yorkshire 4. At Teesdale 1A, the densities of active invertebrates changed little on the control area but dropped significantly (-37%) on the disposal site after sheep dip application ($\chi^2 = 71.9$, $p<0.05$). Sedentary arthropod densities at Teesdale 1A increased significantly (59%) between pre and post disposal sampling in the control area ($\chi^2 = 34.5$, $p<0.05$) and also increased significantly (35%) in the disposal area ($\chi^2 = 17.8$, $p<0.05$). Neither active nor sedentary arthropods differed in density between control and disposal areas at the remaining sites (Table 3.7). The non-arthropod group (molluscs and earthworms) was significantly less abundant on the dip disposal area at Teesdale 1 but more abundant on the disposal area at Wales 2.

Table 3.6: Comparison of geometric mean densities of beetles, flies and tipulid larvae from soil samples within control and sheep dip disposal Sites in 2000.

Site	Date	Dip Type		Beetles geometric mean /m^2	Beetles t	Beetles df	Beetles P	Flies geometric mean /m^2	Flies t	Flies df	Flies P	Tipulid Larvae geometric mean /m^2	Tipulid Larvae t	Tipulid Larvae df	Tipulid Larvae P
Derwent	04/05/00	OP	Control	406.86	2.2	22	<0.05	570.45	4.49	22	<0.01	136.49	0.25	22	NS
		SP	Disposal	262.53				170.12				99.92			
Teesdale1	18/04/00	OP	Control	243.27	2.7	22	<0.05	153.47	1.17	22	NS	83.59	-0.81	22	NS
			Disposal	85.88				104.49				119.18			
Teesdale 1A Pre-disposal	15/05/00	OP	Control	243.59	-0.68	22	NS	231.84	-2.84	22	<0.01	40.82	-2.41	22	<0.05
			Disposal	274.94				758.86				83.59			
Teesdale 1A Post-disposal	11/07/00	OP	Control	239.02	0.47	22	NS	390.86	0.1	22	NS	254.69	-1.42	22	NS
			Disposal	229.55				423.51				282.45			
Teesdale 2	18/04/00	OP	Control	140.41	-1.92	22	NS	296.49	1.81	22	NS	117.22	-0.5	22	NS
			Disposal	334.69				235.76				124.73			
Yorkshire 3	18/05/00	SP	Control	394.45	3.08	22	<0.01	556.41	2.4	22	<0.05	51.92	1.06	22	NS
			Disposal	211.27				293.88				43.43			
Yorkshire 4	02/06/00	SP	Control	353.63	3.78	22	<0.01	432.65	5.11	22	<0.01	177.63	4.4	22	<0.01
			Disposal	140.41				122.12				43.43			
Wales 2	24/05/00	SP	Control	401.96	-1.79	22	NS	248.16	-1.24	22	NS	37.88	0.59	22	NS
			Disposal	486.53				169.47				35.27			
Wales 3	24/05/00	SP	Control	125.39	0.65	22	NS	320.33	-0.39	22	NS	57.47	0.61	22	NS
			Disposal	124.08				367.67				51.59			

Beetles: carabids, staphilinids, weevils, other beetles, beetle larvae
Flies: flies and fly larvae, excluding tipulid larvae

NB. Student's t-tests were carried out on log-transformed data

Table 3.7: Comparison of geometric mean densities of active arthropods, more sedentary arthropods/ soil dwellers and non-arthropods in soil samples from control and sheep dip disposal sites in 2000.

Site	Date	Dip Type		Active arthropods (mainly predators) geometric mean /m²	t	df	P	Soil and more sedentary arthropods geometric mean /m²	t	df	P	Non-arthropods geometric mean /m²	t	df	P
Derwent	04/05/00	OP	Control	937.80	3.8	22	<0.01	678.53	3.8	22	<0.01	319.35	-0.1	22	NS
		SP	Disposal	266.78				335.35				294.86			
Teesdale1	18/04/00	OP	Control	170.78	0.85	22	NS	300.41	1.93	22	NS	175.35	3.44	22	<0.01
			Disposal	109.39				196.24				74.12			
Teesdale 1A Pre-disposal	15/05/00	OP	Control	500.57	-1.56	22	NS	254.69	-1.5	22	NS	46.37	-2.04	22	<0.05
			Disposal	843.10				332.41				92.41			
Teesdale 1A Post-disposal	11/07/00	OP	Control	491.76	0.34	22	NS	405.55	-0.95	22	NS	0.00	0	22	NS
			Disposal	528.98				450.61				0.00			
Teesdale 2	18/04/00	OP	Control	364.73	1.14	22	NS	295.18	-0.96	22	NS	118.20	1.69	22	NS
			Disposal	386.29				396.08				85.88			
Yorkshire 3	18/05/00	SP	Control	632.16	2.38	22	<0.05	555.43	3.96	22	<0.01	0.00	0	22	NS
			Disposal	336.98				220.08				0.00			
Yorkshire 4	02/06/00	SP	Control	749.06	6.93	22	<0.01	462.04	5.45	22	<0.01	0.00	0	22	NS
			Disposal	151.00				132.24				0.00			
Wales 2	24/05/00	SP	Control	530.29	-1.23	22	NS	402.94	-1.99	22	NS	40.82	-3.01	22	<0.01
			Disposal	1001.80				626.61				90.12			
Wales 3	24/05/00	SP	Control	732.41	-0.11	22	NS	277.88	-1.8	22	NS	32.65	-1	22	NS
			Disposal	576.65				461.06				43.43			

Active Arthropods (mainly predators): carabids, staphilinids, other beetle adults, flies, ants, other hymenoptera, spiders, harvestmen, centipedes
Soil and more sedentary arthropods: weevils, beetle larvae, tipulid larvae, all other fly larvae, hemiptera, lepidoptera and sawfly caterpillars
Non-arthropods: earthworms, slugs and snails.

NB. Sudent's t-tests were carried out on log-transformed data

3.3.2 Summary of Soil Sampling Results

The results of the soil sample comparisons are summarised for the two years in Table 3.8 and Figures 3.2 and 3.3, and show that on 7 of 15 sampling occasions significantly lower densities of arthropods were found on the disposal area compared to the control. However, at Teesdale 2 sedentary arthropods were at higher densities on the disposal area, which had a higher organic content than the control, in autumn 1999. The difference between the disposal area and the new control area was not significant in spring 2000. The three sites where it was known that multiple disposals had been made all showed significant density reductions on the disposal areas. Derwent, the "worst case" had significantly reduced numbers of both active and sedentary invertebrates in autumn 1999 and showed no recovery in spring 2000, despite the lack of disposal over winter. Yorkshire 4, which had been used as a disposal site for 30 years, showed a similar lack of recovery in spring 2000. However, Teesdale 1, where the disposal area had been used for 5 years, showed no significant difference in spring 2000 although there was significant reduction in sedentary arthropods after autumn disposal. Despite the variation between sites, comparison between control and disposal areas for all the farms showed a significant 32% reduction in the densities of total invertebrates on the disposal areas in comparison to the controls (paired $t_{14} = 2.18$, $p<0.05$).

There were no significant differences between the densities of worms on disposal and control areas and no overall significant difference comparing the farm differences (Table 3.9).

Table 3.8: Soil sample summary, comparing percentage differences in mean densities of active and sedentary arthropods on dip disposal and control areas, - denotes lower on disposal area, * p<0.05, ** p<0.01)

Site	Autumn 1999		Spring 2000	
	Active	Sedentary	Active	Sedentary
Wales 1	-6	-63	-	-
Wales 2	-25	-5	+89	+55
Wales 3	-	-	-21	+66
Yorkshire 1	-39	-92*	-	-
Yorkshire 2	+35	+40	-	-
Yorkshire 3	-	-	-47*	-60**
Yorkshire 4	-	-	-80**	-71**
Teesdale 1	-34	-69**	-36	-35
Teesdale 1A†	-	-	-37*	+35
Teesdale 2	+59	+81*	+6	+34
Derwent	-50**	-61**	-72**	-51**

† Teesdale 1A comparison is between pre- and post-disposal samples

Figure 3.2: Percentage differences in mean densities of active and sedentary arthropods on dip disposal and control areas, autumn 1999 (negative values indicate greater densities on disposal areas).

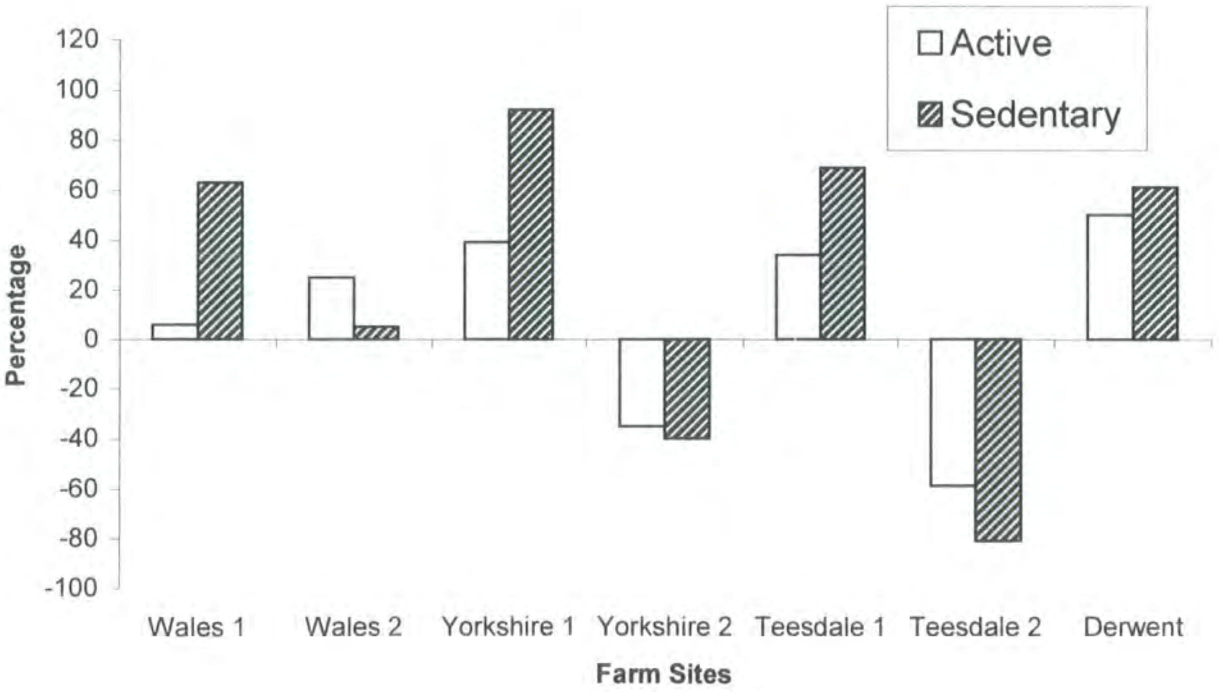
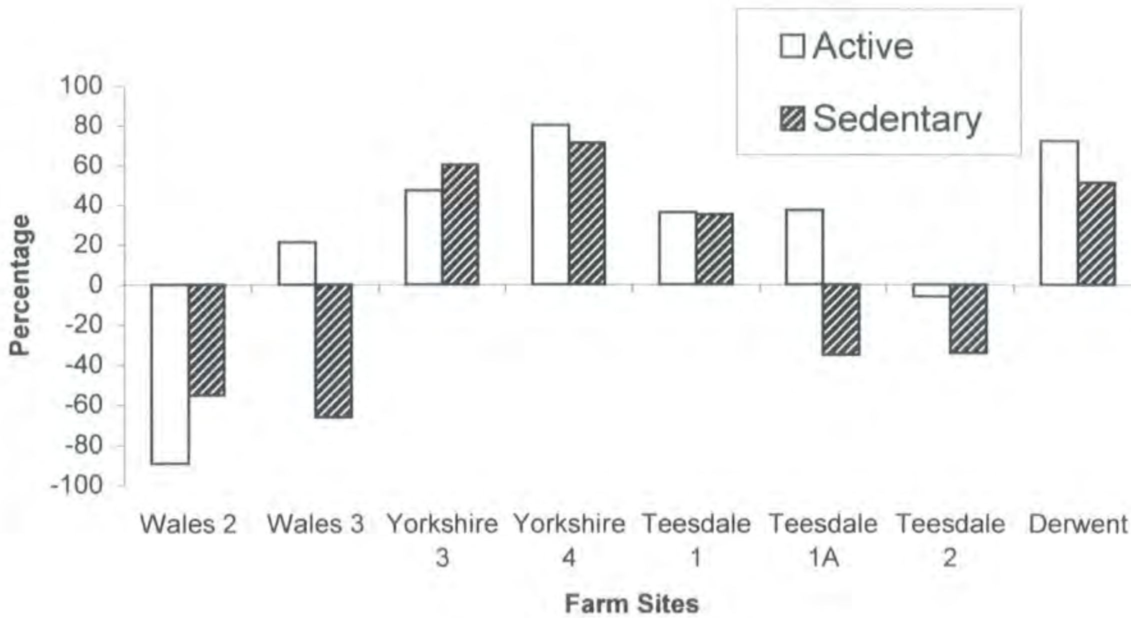


Figure 3.3: Percentage differences in mean densities of active and sedentary arthropods on dip disposal and control areas, spring 2000 (negative values indicate greater densities on disposal areas).



3.3.2.1 Surface active invertebrates

The mean numbers of invertebrates caught in pitfalls were similar on disposal and control areas and did not differ significantly in a paired comparison for all the farms (Table 3.9). The numbers of surface-active invertebrates taken by suction samples were also similar on disposal and control areas although the sedentary arthropods, which were caught in smaller numbers on the disposal areas, were close to the 0.05 significance level (paired $t_9 = 2.09$, $p<0.1>0.05$) (Table 3.9).

Table 3.9 Paired t-tests comparing bird counts and total numbers of invertebrates caught using the different sampling methods found in control and disposal areas in all the study sites 1999-2000

Organisms sampled and method	Geometric mean		t-tests for total numbers of invertebrates			
	Control	Disposal	t	df	P	Sig
total soil invertebrates (Berlese extraction)	27.3 (25-30)	18.8 (16-21)	2.18	14	<0.05	*
surface invertebrates (totals) (suction sampling)	91.8 (89-94)	88.9 (87-91)	0.59	9	>0.05	NS
surface invertebrates (active) (suction sampling)	73.5 (72-75)	78 (76-80)	1.08	9	>0.05	NS
surface invertebrates (sedentary) (suction sampling)	15.3 (13-17)	9.6 (8-12)	2.09	9	>0.05	NS
worms (totals/m^2) (hand sorting)	270.5 (268-273)	280.1 (278-282)	0.86	3	>0.05	NS
surface active invertebrates (pitfall samples)	1126.2 (1124-1128)	1232.8 (1231-1235)	0.19	9	>0.05	NS
birds (area counts)	19 (17-21)	17 (15-19)	1.25	10	>0.05	NS

95% confidence limits are given in parentheses
$P < 0.05$ = Significant

3.3.2.2 Bird counts

A range of bird species was recorded utilising disposal areas including curlew, lapwing, golden plover and redshank (Table 3.10). Both direct and indirect toxic effects of sheep dip disposal could possibly affect these protected species. There were no significant differences between bird numbers using control and disposal areas on any of the study sites or overall (Table 3.9).

Table 3.10: Birds recorded on matching control and disposal sites in Teesdale and Yorkshire in 2000

	T1		T1A		T2		T3		T4		T5		T6		Y2		Y3		Y5		Y6	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
Wading Birds																						
Curlew	-	-	1	0	1	2	-	-	2	1	1	0	0	1	-	-	0	1	-	-	1	0
Golden Plover	-	-	0	2	1	2	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lapwing	20	24	13	3	15	13	8	4	10	2	16	8	9	12	1	1	12	11	4	3	5	2
Oyster Catcher	-	-	-	-	7	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Redshank	-	-	0	2	6	3	5	3	0	2	-	-	-	-	-	-	-	-	-	-	-	-
Snipe	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	20	24	14	7	31	30	13	8	12	5	17	8	9	13	1	1	12	12	4	3	6	2
Geometric means (per site)	3	4	3	2	5	5	3	2	3	2	3	2	2	3	1	1	3	3	2	1	2	1
Other Birds																						
Total	0	0	17	9	36	35	16	10	15	7	20	10	11	16	0	0	0	0	0	0	0	0
Geometric means (per site)	0	0	1	2	1	1	2	1	1	2	1	1	2	2	3	4	1	2	2	2	2	2

3.4 Discussion

Combining the results from all the farms, a significant 32% decrease in invertebrate densities was detected in the soil samples from the disposal areas compared to the controls. However, all the samples contributing to this figure were not independent (four of the farms were sampled in both autumn and spring) and the results from individual farms were highly variable. Teesdale 2 had significantly higher total densities of invertebrates on the disposal area in autumn 1999 and there were other instances where one or more invertebrate groups were sampled in significantly higher numbers on the disposal areas than on the controls. These examples of an apparently positive response to insecticide application could be the result of rapid recolonisation after insecticide use (Jepson, 1989). However, there is no consistent pattern of progressive disappearance of significant effects with time (Tables 3.2 and 3.5) and the source of variation is more likely to lie in sampling error due to the distribution of invertebrates, which tends to be non-random and patchy (Wallwork, 1976).

There were many sources of variability in the use of the historic site information to determine any relationship between disposal activity and invertebrate abundance. Some of these are discussed in relation to the preliminary questionnaire. The size of disposal area varies greatly between farms, depending on the amount of dip to be disposed and rate of application from machinery (Section 3.2.2). Rate of recolonisation is affected by the size of the plot used for pesticide disposal and can lessen the duration of effect on smaller plots (Pullen *et al.*, 1992). In particular, dispersive invertebrate groups such as carabids and staphylinids are only likely to be affected in larger scale disposal (Jepson, 1989). Therefore, recolonisation rates are likely to differ between the farms. Other sources of variability included the interval between dip disposal and sampling and saturation of the soil at the time of disposal. It was not possible to measure the latter but it is likely to influence the rate at which dip penetrates the soil. Rainfall post disposal would also contribute to this as percolating rainwater aids the transport of pesticide residues (Wallwork, 1976).

The farm soil survey has also demonstrated the problems associated with sampling after an event has occurred. The control and disposal areas were assumed to have similar invertebrate populations before dip disposal. Analyses of pH and organic content (Table 3.1) as well as qualitative analysis were used to confirm the disposal and control areas were comparable, but a range of other factors could lead to variability between fields in invertebrate populations. For example, sampling pre-disposal at Teesdale 1A revealed a significantly greater invertebrate density on the disposal site than on the control, despite having similar pH and organic content. This could then be accounted for in the statistical analysis. Such pre-treatment differences would not have been apparent on the other sites with no pre-sampling and the *post hoc* sampling, could therefore have under or overestimated the effect of dip disposal. This highlights the problem of finding comparable control and disposal areas within the same farm when apparently similar individual fields have such pronounced differences in invertebrate fauna.

The most important source of variation, however, may have been in the location of the sampling area relative to the disposal area. On some sites, e.g. Wales 1 and Teesdale 2, the investigator was not confident that the area of disposal had been sampled while on others, such as Wales 2; the dip was bucketed on to the disposal area creating patches. From the point of view of assessing effects of dip disposal on the invertebrate diet for birds, this uncertainty about the disposal area suggests that the 32% average reduction on the disposal sites may be an under estimate of the actual reduction on areas which have received dip.

Fields, which had been used for multiple disposals, or over many consecutive years (Derwent and Yorkshire 4), showed greater decreases in invertebrate densities (55 - 80%), which were still apparent six months after dip disposal. This result is in agreement with the larger scale SCARAB study at Boxworth, which also concluded that repeated use of organophosphate based pesticides in successive seasons can lead to long term declines in abundance of certain arthropods (Frampton, 2001). Although adult birds are unlikely to be affected by decreases of invertebrates within the relatively small areas represented by the disposal plots, decreases of this extent could contribute to pre-fledging mortality for the less mobile chicks. In the first week after hatching, wader chicks move short distances only and are dependent on invertebrates that are either on, or just below, the soil surface (Baines 1990; Galbraith *et al.*, 1993; Whittingham *et al.*, 2001).

No reduction in earthworm densities was observed on any of the disposal sites. In particular, the "worst-case" site at Derwent showed no significant difference between disposal and control area and it is concluded that the disposal of correctly diluted Cypermethrin and Diazinon is unlikely to have adverse effects on earthworm population densities. Studies of the effects of similar pesticides on natural earthworm populations support this conclusion (O'Halloran *et al.*, 1999, for organophosphate based pesticide; Edwards and Brown, 1982, for synthetic pyrethroid studies).

Earthworms comprise a large proportion of the diet of adult lapwing in early spring (Baines, 1990), which suggests that the pre-nesting food supply will be relatively unaffected by dip disposal. However, later in the season adult lapwing take surface-active arthropods, as do other adult waders and chicks of all species (Baines, 1990; Galbraith *et al.*, 1993; Whittingham *et al.*, 2001). These birds may be at risk from depletion of their food supply and of direct exposure to treated invertebrates. The results of the spring bird counts show that the historic farm sites are used by many bird species, including lapwings, golden plover and curlews. Birds fed on disposal as well as control areas and thus could be exposed to contaminated prey on fields where there is spring sheep dip disposal. Direct toxic affects of dip disposal on the relatively small disposal areas are unlikely to cause large scale bird mortality. However, sublethal effects of organophosphates have been shown to alter feeding behaviour of birds, thereby endangering breeding efficiency (Nicolaus and Lee, 1999) and detrimental effects of synthetic pyrethroid pesticides on the health of wild passerines have also been noted (Bishop *et al.*, 1998). Pesticide induced reduction in food availability may also contribute to breeding failure by increasing the foraging time required to fulfil the dietary requirements of chicks (Park *et al.*, 2001), exposing young broods to harsh upland environments for longer or leaving the clutch open to predation, a major cause of breeding failure (Baines, 1990; Grant *et al.*, 1999). The invertebrate groups affected by dip disposal included abundant groups, e.g. beetles and flies, and sedentary arthropods, such as tipulid larvae, which all contribute to the avian diet.

The many potential sources of error encountered in the farm site study mean that the results can only be viewed as indicative. However, total invertebrate densities, on the disposal areas as a whole, were significantly lower and were about two thirds of the densities on the control areas. Both active and sedentary groups showed reductions and it can be assumed that recolonisation on the relatively large areas used for disposal at the farm sites is slow, even for active invertebrates. In spring 2000 invertebrate densities on three of the disposal areas were still significantly below the control areas following disposal in autumn 1999, with densities on the two sites that had been used for long-term disposal particularly depressed.

Although adult waders can move to new areas if they find one foraging area unprofitable, this may not be possible for young chicks. Invertebrate reductions of 55-80%, found at Derwent and Yorkshire 4, could impose severe restrictions on growth in the first few days when chicks can move short distances only.

4. INVERTEBRATE SPECIES RESPONSE - ORDINATION OF PITFALL CATCHES

4.1 Introduction

Pitfall catches from the paired disposal and control areas on the farm sites sampled in 2000 (Chapter 3, Table 3.1) provided data for the investigation of invertebrate species response to sheep dip disposal. There was no indication from the numbers of invertebrates caught in pitfall traps that dip disposal had had any effect on the combined abundance and activity of the surface active invertebrates (Table 3.9). However, pitfall traps capture invertebrates from an unknown area and give comparative abundance estimates only (Southwood and Henderson, 2000). Pitfall samples have therefore not been used to provide density comparisons but to provide large numbers of individuals to allow comparison of community composition at different sites.

Sheep dip disposal could have had differential effects on the survival of different species and altered community structure. Simpson's Diversity Index (D) (Krebs, 2001) was used to compare species diversity on control and disposal sites and CANOnical Community Ordination analysis or CANOCO (Ter Braak, 1988) was carried out on the spider and ground beetle assemblages. "Canonical ordination is a combination of ordination and multiple regression. This leads to an ordination diagram of samples, species and environmental variables, which optimally displays how community composition varies with the environment" (Ter Braak, 1988). The analysis quantifies the relative importance of the environmental variables contributing to the major axes of variation and is, therefore, an appropriate tool for identifying the effects of perturbations such as dip disposal.

A CANOCO ordination represents continuous change of species composition along the environmental gradients, represented by the ordination axes. The species distributions are constrained by the measured environmental parameters and interpretation relies on the idea that proximity implies similarity (Leps and Smilauer, 2003). Therefore, sites with similar species compositions have similar axis scores and are depicted close together and when species distributions are plotted, species that occur together on the same sites have similar axis scores. Species that do not occur together are widely separated within the ordination space. Axis 1 represents the best fit, for both species and environmental variables, and comparison of eigenvalues indicates the relative power of the other axes. Environmental variables, contributing significantly to the axes, are represented by arrows on the diagrams. The length of the arrow (direction in which the value of the environmental variable increases) indicates the degree of influence of the environmental variable on the species distribution. Sites close to an arrow head on the ordination are particularly associated with that variable. The angle between arrow and axis indicates its importance to the axis. The significance of the relationship between species distribution and the environmental variables can be tested using the Monte Carlo permutation test (Ter Braak, 1988; Leps and Smilauer, 2003).

4.2 Methods

Pitfall sampling methods for collection of the raw data are detailed in Chapter 3. The total species assemblage of spiders and of ground beetles from pitfall trap catches at the farm sites were analysed as two separate groups using CANOCO (Ter Braak, 1988). Numbers of individual species were log transformed and sample scores were calculated as weighted mean species scores. Single occurrences of spider species at a site were ignored and rare species were down-weighted for both spiders and beetles. The environmental variables entered in the analyses included: altitude, slope, pH, organic content of the soil and presence of sheep dip.

Field management was entered as a series of nominal variables: pasture, improved grazing and hay meadow. Region (either Yorkshire or Teesdale) was added as a covariable because the effect of region was not intended for interpretation but needed to be taken into account when judging the effects of the other variables. With the exception of Teesdale 1A, where dip was disposed for the first time in spring 2000, all sites received their last application of dip in autumn 1999 and the timing of dip disposal has not been entered as a variable.

A series of canonical ordinations were produced to display the multivariate data analysis. Separate ordinations were created for spiders and carabids to retain clarity since many species were involved. Detrended Correspondence Analyses (DCAs) on the spider and carabid assemblages were carried out prior to Canonical Correspondence Analysis (CCAs). The DCA is an unconstrained ordination that provides a "basic overview of the compositional gradients in the data" (Leps and Smilauer, 2003) and must be carried out before the CCA to establish the main variability in species composition that is not related to the environmental variables. CCA produces constrained ordinations to show the variation that is related to the measured environmental variables. CCA ordinations were also produced to examine species assemblages for beetles and spiders.

As the species are quite numerous (59 spider species and 39 beetles species) it is impossible to show each species name on the ordination and retain clarity. Therefore, spiders that exhibit similar behaviour have been joined into foraging guilds according to web structure or time of activity (Post and Riechert, 1977) to aid the interpretation of the distribution of spider species across the ordination. The spider species are categorised into the following foraging guilds: Funnel Web Spiders, Sheet Line Weavers, Diurnal Running Spiders, Orb Weavers, Scattered Line Weavers and Crab Spiders. Key beetle species, that are indicators of particular habitat types, are named on the ordination. Others beetles are represented by data points and indicative comments are included about the habitat type in different areas of the graph. A full list of the spider and carabid species is given in Appendix 2.

4.3 Results

4.3.1 Spiders

The first gradient in the DCA is the longest (Table 4.1) and therefore explains more about the total species variability than the second axis. The eigenvalue of the first axis is almost double that of the second axis. The first axis is also quite well correlated with the environmental data (r = 0.896), with lower correlation on the second axis (r = 0.644).

Table 4.1: DCA and CCA results based on spider assemblages

	DCA		CCA	
Axes	1	2	1	2
Eigenvalues :	0.318	0.161	0.172	0.12
Lengths of gradient :	2.314	1.518		
Species-environment correlations :	0.896	0.644	0.906	0.937
Cumulative percentage variance				
of species data :	25.4	38.3	11.7	19.8
of species-environment relation:	45.2	54.9	32	54.2

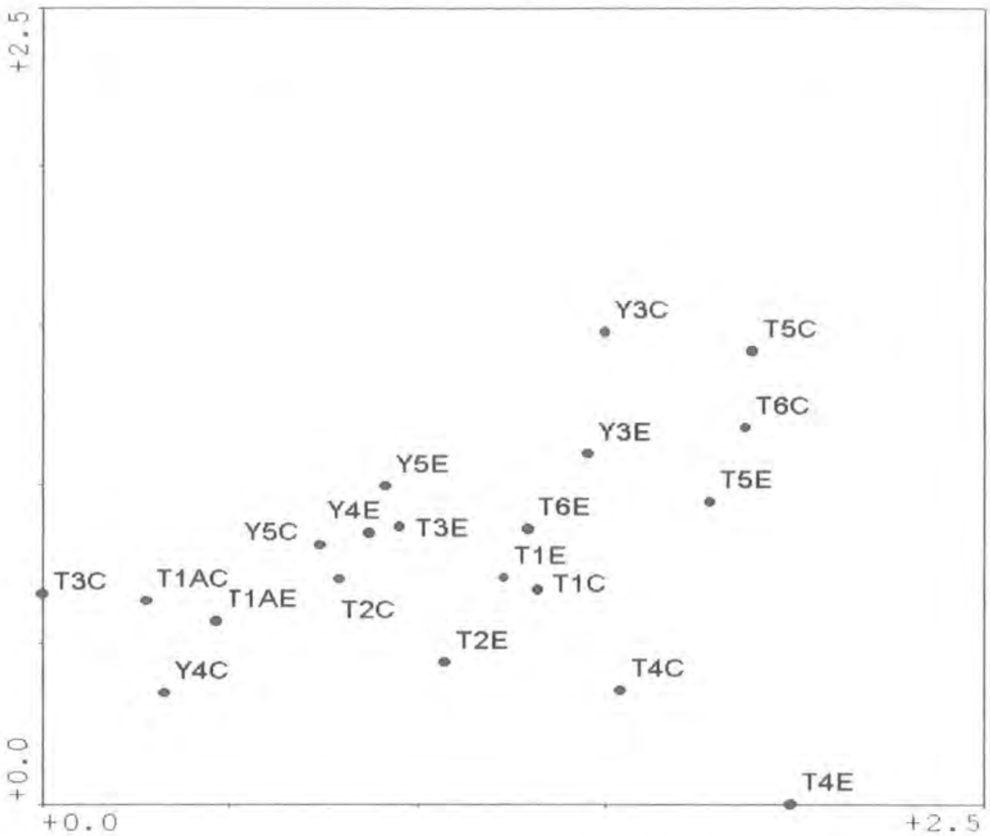


Figure 4.1: DCA Ordination of the farm sites using axis 1 against axis 2 based on spider assemblages at farm sites in Yorkshire (Y) and Teesdale (T) in 2000 (Table 3.1)

(C = control area, E = disposal area)

The unconstrained DCA ordination (Figure 4.1) indicates there are differences in community composition at the farm sites by the positioning of sites across the ordination. On both the DCA and CCA ordinations (Figures 4.1 and 4.2) the sites are lying in approximately the same relationship to each other e.g. T5C is at the opposite side of the ordination to T3C in both cases but the distribution is transposed on the CCA. The CCA indicates that the species distribution of spiders was not significantly related to the environmental variables on axis 1 at more than the 90% level (Monte Carlo permutation test, $p<0.1$). The t-values therefore have little exploratory value. Land use was the variable most likely to have influenced distribution, with the highest canonical coefficient (1.28) and exploratory t-value (3.97) for axis 1 (Table 4.2). Slope made the second strongest contribution with a canonical coefficient of -0.49 and an exploratory t-value of -2.59. The percentage variance explained by the first axis in the CCA is about half that explained by the first axis in the unconstrained DCA (11.7 in comparison with 25.4) (Table 4.1) and the species-environment correlation is considerably higher in the DCA.

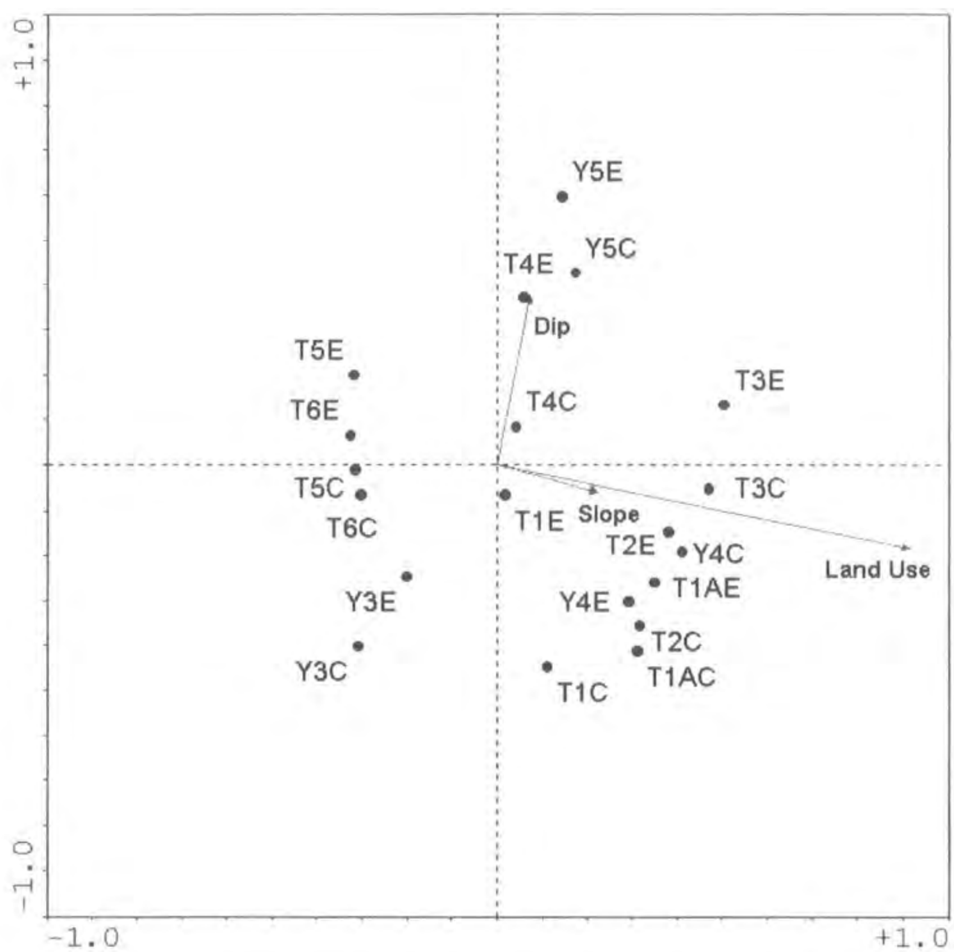


Figure 4.2: CCA Ordination of the farm sites using axis 1 against axis 2 based on spider assemblages at farm sites in Yorkshire (Y) and Teesdale (T) in 2000 (Table 3.1)

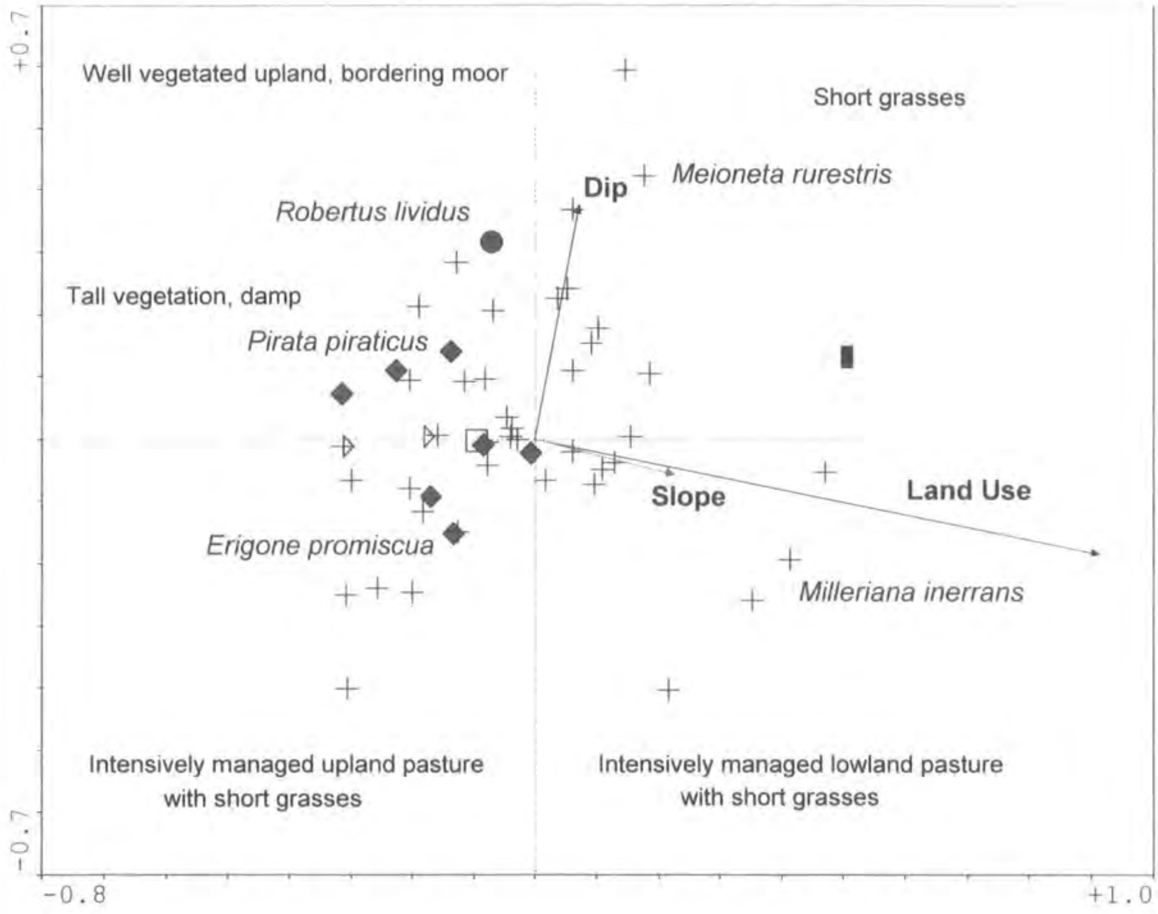
(C = control area, E = disposal area)

The Monte Carlo permutation test for all the axes combined indicates that the distribution of the species was not significantly correlated with the variables. This suggests that while the measured variables land use and slope may influence spider community composition, the major influence was from a variable that was not measured. The relatively low eigen values in this model support this explanation. Although dip has a high t-value for axis 2 (Table 4.2) it is not a significant factor. However, at all the farm sites except Yorkshire 4 (Y4) the disposal areas are displaced upwards on the CCA ordination (Figure 4.2) in comparison with the control sites, a trend that can be attributed to dip disposal.

Table 4.2. Eigenvalues and canonical coefficients (with "t" values) for the first two axes of CCA analyses on spider species assemblages, caught in pitfall traps, on control and disposal areas at the farm sites

	Spiders CCA			
	Axis 1	t	Axis 2	t
Eigenvalue	0.172		0.12	
Cannonical coefficients				
Dip	0.00	0.01	0.34	3.09
Land Use	1.28	3.97	-0.05	-0.21
pH	0.41	1.29	0.74	2.95
Organic Content	0.08	0.41	-0.53	-3.34
Slope	-0.49	-2.59	-0.14	-0.92
Altitude	0.73	0.92	2.26	3.58

Figure 4.3 shows the CCA distribution of the six foraging guilds of spiders that are represented by the species captured at the farm sites and indicates groupings of spiders with similar habitat requirements. Families represented by these foraging guilds are given in Table 4.3 and species scores are given in Appendix 2.



Foraging guilds are shown; Funnel Web Spiders: rectangle, Sheet Line Weavers: cross, Diurnal Running Spiders: diamond, Orb Weavers: square, Scattered Line Weavers: Circle, Crab Spiders: triangle.

Figure 4.3: CCA Ordination of spider assemblages, using axis 1 against axis 2, showing typical habitat characteristics of key species at farm sites in 2000

Table 4.3: Spider Families represented in the Foraging Guilds of spiders caught at farm sites in 2000

Foraging Guilds	Family
Scattered Line Weavers	Theridiidae
Orb Weavers	Tetragnathidae
Sheet Line Weavers	Linyphiidae
Funnel Web Spiders	Amaurobiidae
Diurnal Running Spiders	Lycosidae
Crab Spiders	Thomisidae

The sheet line weavers are the most represented guild and are distributed across the ordination. Only one species from each of the funnel web spiders, orb weavers and scattered line weavers is represented, which limits their usefulness in interpretation. However, the diurnal running spiders and crab spiders are grouped together on the left of the ordination, towards the positioning of farm sites Teesdale 5 and 6 and Yorkshire 3 (Figures 4.2 and 4.3 and site descriptions, Chapter 3). To the upper left area of the ordination are species that are commonly found in well vegetated upland areas, close to moorland such as *Robertus lividus* and *Pirata piraticus* which prefers damp environments (Rushton and Eyre, 1992; Cherret, 1964). On the lower left area of the ordination are species that are commonly found in short grasses in upland areas such as *Erigone promiscua* and on the right hand site are species found on intensively managed lowland areas with short grasses such as *Meioneta rurestris* and *Milleriana inerrans* (Rushton and Eyre, 1992).

4.3.2 Ground beetles

The first gradient is the longest in the DCA for ground beetle assemblage (Table 4.4) and therefore explains more about the total species variability than the second axis. The first axis is also well correlated with the environmental data (r = 0.925), with only slightly lower correlation on the second axis (r = 0.905).

Table 4.4: DCA and CCA results based on ground beetle assemblages

	DCA		CCA	
Axes	1	2	1	2
Eigenvalues :	0.718	0.283	0.407	0.278
Lengths of gradient :	3.878	2.487		
Species-environment correlations :	0.925	0.905	0.982	0.948
Cumulative percentage variance				
of species data :	20.9	29.1	21.2	35.7
of species-environment relation:	30.7	48.9	43.2	72.7

The unconstrained DCA ordination (Figure 4.4) indicates there are differences in ground beetle community composition at the farm sites but despite inputting the covariable the sites still separate out along axis 1 according to region to some degree. In the DCA and the CCA ordinations (Figures 4.4 and 4.5) the sites are lying in the same relationship to each other, with the exception of Y3C and Y3E but the orientation is turned 90° anticlockwise in the CCA. The percentage variance explained by the first axis in the CCA is very close to that explained by the first axis in the unconstrained DCA (21.2 in comparison with 20.9) and the species-environment correlation is moderately higher in the CCA (Table 4.4), indicating that the measured variables influence species community composition. The CCA indicates that the distribution of carabid species along axis 1 was highly significant (Monte Carlo permutation test, $p<0.005$). A Monte Carlo permutation test on the combined axes was also highly significant ($p<0.005$). Therefore it can be accepted that the included environmental variables explain a significant proportion of the variation in ground beetle community composition.

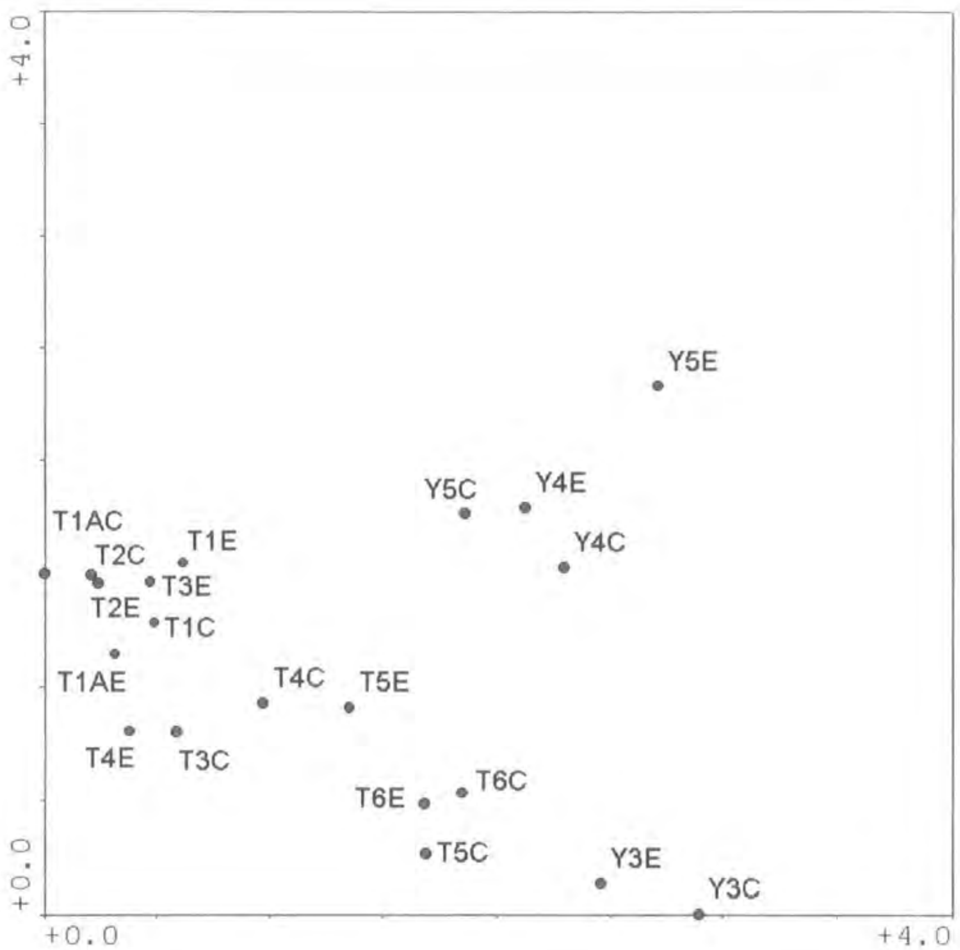


Figure 4.4: DCA Ordination of the farm sites using axis 1 against axis 2 based on ground beetle assemblages at farm sites in Yorkshire (Y) and Teesdale (T) in 2000 (Table 3.1)

(C = control area, E = disposal area)

Land use and altitude were the most important influences on carabid distribution with axis 1 canonical coefficient scores of –1.33 for land use (exploratory t = -9.85) and -1.25 for altitude (exploratory t = -4) (Table 4.5). The canonical score of -0.14 for dip application (exploratory t = -2.41) also indicates the possibility of a small influence of dip disposal on axis 1. In addition, apart from Yorkshire 3 (Y3) and Yorkshire (4) the disposal sites are displaced upwards on the ordination compared to the control sites, indicating a possible influence of dip disposal (Figure 4.5). Land use also made an important contribution to axis 2 but the greater influence of organic content is indicated with a score of –1.01 (exploratory $t = -5.97$). Again, a canonical score of 0.25 (exploratory $t = 2.58$) indicates that dip application has an influence on axis 2.

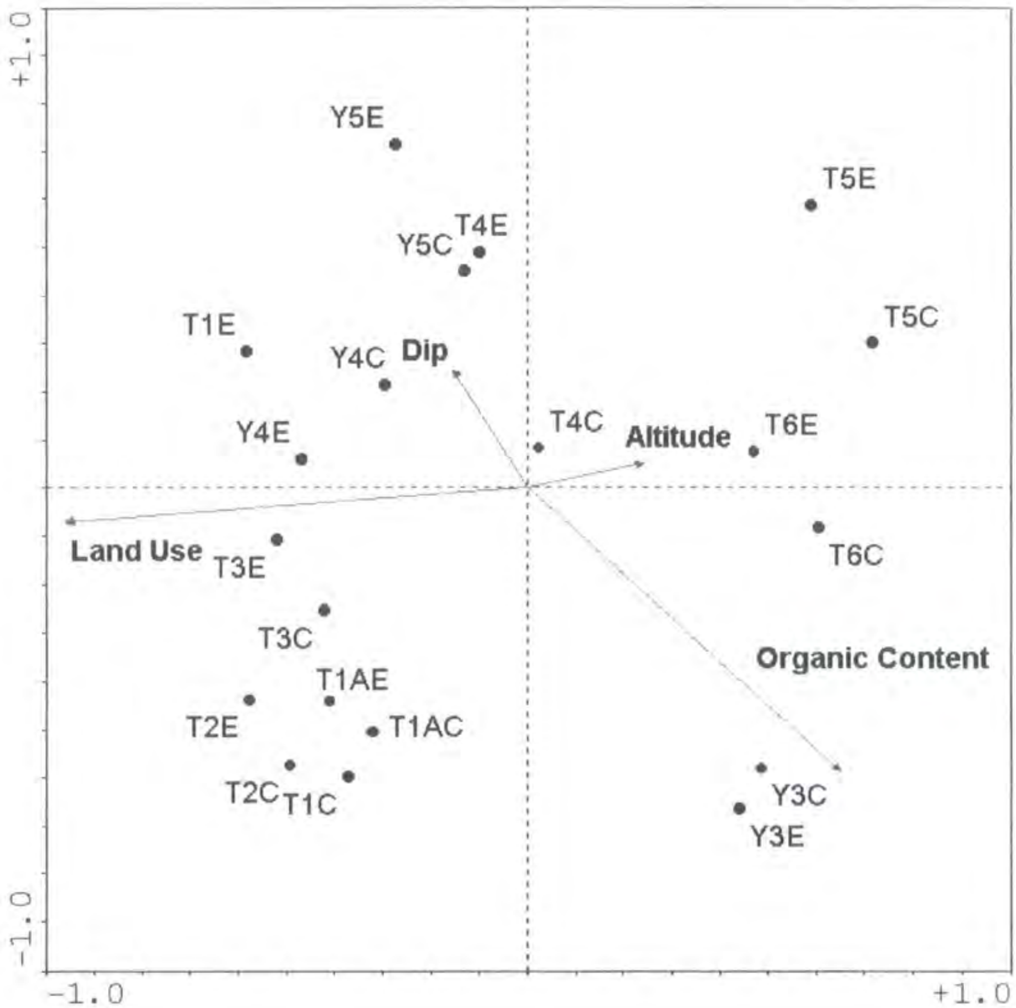


Figure 4.5: CCA Ordination of the farm sites using axis 1 against axis 2 based on ground beetle assemblages at farm sites in Yorkshire (Y) and Teesdale (T) in 2000 (Table 3.1)

(C = control area, E = disposal area)

Table 4.5. Eigenvalues and canonical coefficients (with "t" values) for the first two axes of CCA analyses of ground beetle species assemblages, caught in pitfall traps, on control and disposal areas at the farm sites

	Ground beetles CCA			
	Axis 1	t	Axis 2	t
Eigenvalue	0.407		0.278	
Cannonical coefficients				
Dip	-0.14	-2.41	0.25	2.58
Land Use	-1.33	-9.85	-1.06	-4.51
pH	0.13	1.00	0.47	2.02
Organic Content	0.09	0.90	-1.01	-5.97
Slope	-0.02	-0.25	0.25	1.93
Altitude	-1.25	-4	-0.05	-0.09

The CCA ordination of ground beetle assemblages (Figure 4.6) indicates groupings of beetles with similar habitat requirements. Species scores are given in Appendix 2. To the left of the ordination are species that are commonly found in lowland, improved grasslands such as *Pterostichus melanarius* (Luff *et al*, 1992), which corresponds with the characteristics of farm sites (including Teesdale 1, 2 and 3, which occur along the lower slopes of the Tees Valley) plotted in that area in the ordination of farm sites (Figures 4.5 and 4.6 and site descriptions, Chapter 3). To the right of the ordination are species such as *Carabus problematicus* and *Pterostichus nigrita agg.*, which are found in less intensively farmed or unmanaged upland grasslands that tend to include longer grasses (Luff *et al.*, 1992).

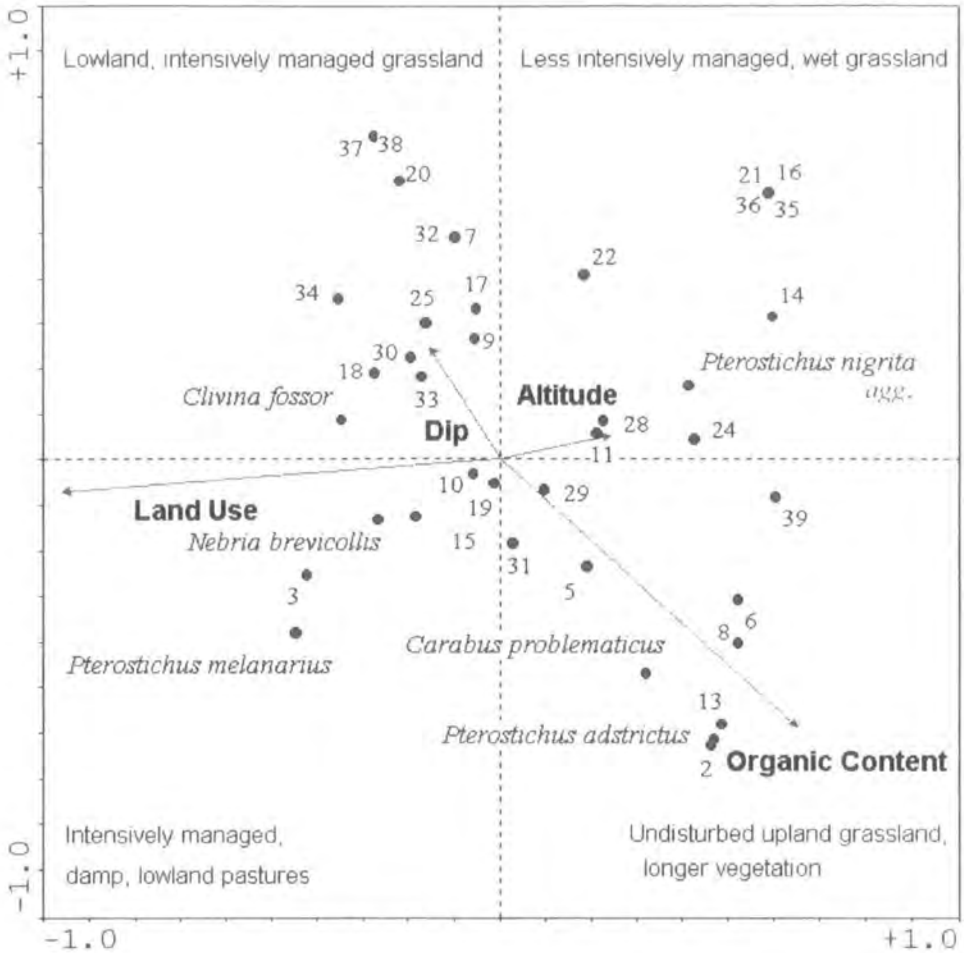


Figure 4.6: CCA Ordination of ground beetle assemblages using axis 1 against axis 2 showing typical habitat characteristics of key species at farm sites in 2000

Comparison between species diversity on control and disposal sites

Simpson's Diversity Index (D) which takes into account both species richness and equitability (Krebs, 2001) showed no significant differences between spider diversities on control and disposal sites (Table 4.6) and diversities on disposal and control sites at each farm were closely correlated ($y = 1.06x - 0.18$, $r_9 = 0.973$, $p<0.001$). Ground beetle diversity, however, was significantly higher on disposal sites than controls, $t_9 = 2.51$, $p< 0.05$ (Table 4.6). This was mainly due to lower numbers caught and higher equitability on the disposal areas, though this trend was not consistent. The relationship between diversities on disposal and control sites at each farm was not significant ($y = 1.01x + 1.16$, $r_9 = 0.437$, n.s.).

Table 4.6 Simpson's diversity indices (D) based on pitfall catches of spiders and ground beetles at the farm sites.

	D (spiders)			D (ground beetles)		
Sites	Control	Disposal	C-D*	Control	Disposal	C-D*
Yorkshire 3	8.57	9.36	-0.79	3.5	6.62	-3.12
Yorkshire 4	2.97	3.94	-0.97	2.11	3.83	-1.72
Yorkshire 5	3.84	2.63	1.21	4.72	7.49	-2.77
Teesdale 1	5.12	4.3	0.82	3.24	2.61	0.63
Teesdale 1A	1.79	1.8	-0.01	3.24	2.27	0.97
Teesdale 2	3.52	4.39	-0.87	1.92	2.99	-1.07
Teesdale 3	3.93	3.91	0.02	1.52	1.33	0.19
Teesdale 4	11	11.6	-0.6	3.25	3.71	-0.46
Teesdale 5	6.34	6.49	-0.15	2.71	8.09	-5.38
Teesdale 6	6.05	6.24	-0.19	1.57	5.54	-3.97
mean difference			-0.153			-1.67
paired t			-0.67n.s.			-2.51 p<0.05

* Difference between control and disposal sites

4.4 Discussion

CANOCO (Ter Braak, 1988) indicated that the effects of dip application on spider assemblages on the farms were negligible and the influences of the other measured environmental variables were not the most significant in determining spider community composition. Vegetation structure was not measured at the farm sites but is considered a major influence on spider distribution (Cherrett, 1964; Coulson and Butterfield, 1986; Downie *et al.*, 1995). The effects of different management regimes on vegetation structure have also been found to strongly influence spider communities (Rushton *et al.*, 1987; Luff and Rushton, 1989; Rushton *et al.*, 1989, Gibson *et al.*, 1992; Rushton and Eyre, 1992). In addition, spider abundance and community diversity may be positively correlated with vegetation density (Duffey, 1962; Cherrett, 1964). It is therefore possible that measuring different aspects of the vegetation cover at the farm sites might have revealed more important influences on spider community composition. The ordination of spider species assemblages at the farm sites (Figure 4.3) shows distribution of species approximately according to habitat types. The clustering of diurnal running spiders to the left of the ordination approximately corresponds with the positioning of farm sites Teesdale 5 and 6 in Figure 4.2. These were less intensively managed sites, close to moorland and incorporating patches of short grazed grasses with long, tussocky grasses such as *Nardus stricta*, with *Juncus* interspersed (Site Descriptions, Chapter 3). The presence of *Pirata piraticus* is indicative of the damp environment associated with these habitat types (Rushton and Eyre, 1992; Cherret, 1964) and the scattered line weaver *Robertus lividus* found in the same area is characteristic of well vegetated upland moors (Rushton and Eyre, 1992). Numbers of diurnal running spiders have been found to be positively correlated with vegetation density and may take advantage of increased prey availability due to structural diversity and plant taxonomic diversity at such sites (White and Hassal, 1994). *Milleriana inerrans* and *Meioneta rurestris*, which are characteristic of intensively managed lowland areas of short grasses (Rushton and Eyre, 1992), are to the right of the ordination (Figure 4.3) approximately corresponding to the placement of farm sites such as Yorkshire 5 and Teesdale 2 that meet this description (Figure 4.2 and site descriptions, Chapter 3). Of all the species represented, *Erigone atra* and *E. dentipalpis* were caught in the greatest numbers and were found on all the farm sites but, in accordance with findings by Rushton and Eyre (1992), were most numerous on sites containing intensively managed short grassland such as Yorkshire 5, (Appendix 2). Land use was the most important of the measured variables on spider community composition in this investigation, which supports the theory that vegetation cover may be the major influence as these variables are closely related.

Although dip application was not found to have a significant influence on spider communities on the farm sites in Teesdale and Yorkshire, other studies have indicated density decreases as a result of similar pesticides. The study on the experimental farms has shown significant reduction in pitfall catches of lycosid spiders after Cypermethrin application (Sourhope Latin Square, Chapter 5) but not of linyphiids. Insecticides, such as Dimethoate, have highly toxic effects on spiders when applied at field dosage rates (Vickerman and Sunderland, 1977) but there is some evidence that the effects of some insecticides on spiders are not as long lasting as on ground beetles. Rushton *et al.* (1989) found Chloropyrifos application was detectable, as a factor within management intensification, acting on the ground beetles but concluded that spiders were probably responding more to the change in vegetation structure. The susceptibility of non-target organisms depends not only on the sensitivity of the species but also on the degree of exposure of the active stages to the insecticide. Plant-active linyphiid spiders were found to be adversely affected by pyrethroid application whereas ground-active species were not, suggesting persistence of the pyrethroid at the plant surface and rapid inactivation in the soil (Brown *et al.*, 1988). Furthermore, in a study of pesticide applications to winter wheat Pullen *et al.* (1992) found that Linyphiidae were profoundly affected by the synthetic pyrethroid, Deltamethrin, showing 168-221 day depletions in the 4 ha plots. The recovery of populations after insecticide application depends largely on the capacity of the species to re-colonise the area, which reduces the duration of effects on dispersive groups. Although linyphiids may succumb to the immediate effects of insecticide, their capacity for rapid recolonisation means that they are at much lower risk of long-term population depletion than carabids as a group (Jepson, 1989). This is supported by the transient depletions of spider populations due to pesticides in other studies (Inglesfield, 1985; Cole *et al.*, 1986). In all but one case (Teesdale 1A) pitfalls were used on farms where dip had been disposed the previous autumn. Spider populations, although they could have been affected in the short-term

(Vickerman and Sunderland, 1977), had probably had time to recover. Pullen *et al.* (1992) found a correlation between plot size and the duration of the effects of pesticide application, particularly associated with Linyphiidae.

In the present study CANOCO (Ter Braak, 1988) suggested that the effects of dip application on ground beetle assemblages on the farms were significantly influencing community composition, although to a lesser extent than altitude and land use. Using a similar multivariate approach Rushton *et al.* (1989) found an obvious decrease in carabid species richness associated with organophosphate (Chloropyrifos) application on large areas (2-11 ha) of upland pasture. A study on the short term impact of Chlorpyrifos and Cypermethrin showed a brief (24hr) increase in numbers of carabids on the treated area, attributed to movement over small plots, followed by a significant decrease for the remainder of the study period (Curtis and Horne, 1995). As Diazinon has been recommended for control of the carabid strawberry pest *Harpalus rufipes* (Briggs and Tew, 1969), OP application was expected to have adverse effects on ground beetle population densities. Further, in another study, Cypermethrin application in spring resulted in reduced catches of ground beetles for about a month while autumn application led to decreased densities of overwintering larvae of the common grassland species *Nebria brevicollis* (Cole *et al.*, 1986). No effects were apparent in the next generation in the following year and it is likely that the plots were re-colonised by this active species. In the present study *N. brevicollis* was caught in significantly higher numbers on the farm disposal areas and, as catches of carabids other than *N. brevicollis* tended to be lower on the disposal areas, this may represent successful invasion of areas where competition has been reduced. On large areas, insecticide application is likely to have longer term adverse affects on the less active species, resulting from their limited ability to re-colonise the area (Rushton *et al.*, 1989; Jepson 1989). As with the Linyphiidae, the dispersal capacity, size and location of reservoir populations near a site of depletion are also key factors in the recovery potential of a given taxon (Thacker and Jepson, 1993).

Comparison of the similarity between spider diversities and the significant differences between ground beetle diversities on disposal and control areas (Table 4.6) suggests dip application affects carabids but not spiders. However, the higher carabid species diversity detected on the disposal sites in this study, which is the opposite of the results found by Vickerman and Sunderland (1977) can be attributed to more than one factor. Land use was the most important variable influencing carabid community composition revealed using CANOCO (Table 4.5). This is supported by other studies that found site management to be a key factor in classifying and predicting habitat groups (Luff *et al.*, 1992; Luff, 1996; Luff *et al.*, 1989). Altitude, which is also important in ground beetle habitat classification (Luff *et al.*, 1992), was the second most important variable on axis 1 of the CCA (Table 4.5; Figure 4.5). Eyre *et al.* (1986) determined that moisture and sand content on soil in particular affected carabid communities and Luff *et al.* (1992) also use soil water and soil bulk density to refine habitat predictions. These variables were not measured in this investigation but could be responsible for small amounts of unexplained variance in the data.

The CCA ordination of ground beetle assemblages at the farm sites (Figure 4.6) shows carabid species separated across the ordination approximately grouped into habitat types as established by Luff *et al.* (1992). Species such as *Carabus problematicus* and *Pterostichus adstrictus* are found in the lower right quadrant of the ordination, which corresponds with the placement of farm sites Yorkshire 3 and Teesdale 6, where they were collected (Figure 4.5). These are upland grassland sites close to moorland and are Habitat 1 in the classification scheme (Luff *et al.*, 1992). The presence of *Clivina fossor* on the left of the ordination corresponds with wet, relatively lowland sites (Habitat 9), which relates to several sites including Yorkshire 4 and Teesdale 3. The influence of dip application has therefore not dramatically altered species assemblages from that which could be expected from the range of habitat types sampled but might be responsible for subtle changes, as indicated by the CCA results (Table 4.5). Although diversities on control and disposal sites were correlated, moderate separation of the control sites from disposal sites in the ordination (Figure 4.5) might indicate the influence of dip application.

The CANOCO analysis indicated a small but significant effect of dip disposal on the carabid species composition of the pitfall catches. The diversity of carabids was higher on the disposal areas than on the controls and the relationships between numbers caught on disposal and control areas at each site were not significant. In contrast, spider species composition and diversity were not significantly related to dip disposal although studies using similar pesticides have shown significant effects. Numbers caught on disposal and control areas were significantly correlated. These differences between carabids and spiders indicate that the disposal of sheep dip has a disrupting effect on species composition in carabid communities but that spiders are less vulnerable. This is partly due to the efficient recolonisation capabilities of many spider species. There were also indications that there are differences in response to insecticide applications at the species level as well as the differences between the major taxa.

5. LATIN SQUARE EXPERIMENT

5.1 Introduction

The farm sites (Chapter 3) gave very useful indications of the effects of sheep dip disposal on non-target organisms on farmland but the invertebrate survey suffered from several problems. These problems included locating the exact area of disposal on some of the farms and difficulties were also encountered in finding appropriate replicate control sites at the farm scale. The purpose of experimental application was to avoid the uncertainties of the farm survey by carrying out a multifactorial replicated plot experiment to investigate the effects of different dilutions of SP and OP dip on invertebrate activity and abundance. The aim was to reflect the real situation on farms as far as possible, using widely available dips and applying the dip at the same dilution and volume per area, as the quantities per hectare specified in EA guidelines. The dilutions used at the experimental sites included made up dip diluted 1:3 with water, as recommended by EA guidelines, and made up dip without any further dilution, as the farm studies showed that the dip was not always diluted for disposal, at least not to specified levels.

The Latin Square design experimental method takes account of any possible environmental gradient effects e.g. slope or drainage. It uses a quadrat grid with equal numbers of columns and rows, set out so that no treatment occurs more than once in any row or column thus avoiding any uncontrollable factor influencing one treatment more than another and providing statistically tenable results. The disadvantage is that available space and manpower dictated a maximum plot size for application of 10 x 10 m. Each treated plot, therefore, represented only a small proportion of the plot size actually used for disposal on farms, the smallest of which was approximately equivalent to the entire experimental plot area. Rates of recolonisation, after any density reductions suffered as a result of dip disposal, will therefore differ, and probably be more rapid, on the Latin square plots than in the real farm situations.

The Before-After/Control-Impact (BACI) sampling method was used and soil invertebrates were sampled using similar methods to those used in the farm sampling (Chapter 3), from each plot in the Latin square prior to dip application and at intervals post application. This allowed changes in population densities of invertebrates to be measured over a precise time period, with known invertebrate densities prior to dip application, which had not previously been possible in most of the farm sites (Chapter 3). Other sampling techniques such as pitfall trapping and suction sampling were also carried out where possible.

Experimental farms at Sourhope and Newton Rigg were chosen for the study using plots in a Latin Square design. The sites at were chosen to compliment each other, allowing assessment of the two main types of farmland used for dip disposal, rough grazing and improved pasture, established in the farm questionnaire (Chapter 2) and farm sampling (Chapter 3). The importance of the timing of dip disposal was also investigated using a spring application at Sourhope and autumn and spring applications at Newton Rigg. The effects of a second application of dip in spring following an autumn application on the same area was also investigated on one of the two Latin Squares used at Newton Rigg.

In addition to the main experiment at Newton Rigg 2002, a small supplementary study was carried out specifically designed to investigate the effects of dip disposal on smaller invertebrates, such as Collembola, which make an important contribution to the diet of some species of carabid beetle (Toft and Bilde, 2002). Results from both farm sites and initial experimental plot work at Sourhope showed that the disposal of both organophosphate and synthetic pyrethroid sheep dips had a detrimental effect on some relatively large invertebrates including adult and larval Coleoptera. These invertebrates feature strongly in the diet of important wading bird species such as golden plover (Ratcliffe, 1976) and lapwing (Baines, 1990) for which several upland SSSIs have been designated, in particular those in Teesdale and Wales where part of the previous research was undertaken. It is therefore also important to know whether the smaller invertebrate prey species of some of the larger predatory species are also affected by dip disposal to farmland. The aim was to determine whether effects of the dip on the larger invertebrates were due simply to direct effects of the dip or whether they could have resulted from indirect effects of a drop in food supply. Long-term negative effects of pesticide regimes have been found previously on collembolan communities sampled by pitfall trapping as part of the SCARAB project on agricultural land (Holland *et al.*, 2002) and in other work on Collembola using suction sampling (Frampton, 2000, 2001).

5.2 Methods

5.2.1 Site Descriptions

Information gathered in the preliminary survey (Chapter 2) indicated that farmers prefer not to use their best quality grazing land for disposal where possible. Vegetation was identified according to Rose (1981). The experimental site at Sourhope (Plate 1) was chosen to represent the type of rough grazing land that farmers typically choose for dip disposal, if it is available to them. Sourhope Experimental Farm (National Grid Ref.: NT845202) is in southern Scotland, twelve miles East of Jedburgh and was used in spring 2000 in an assessment of the short-term, direct effects of dip disposal. The soil at Sourhope had an organic content of approximately 16.3%, a pH of approximately 4.5 and was situated at an altitude of approximately 425m above sea level. The dominant vegetation consisted of grasses, mainly *Nardus stricta* interspersed with *Agrostis* sp and *Festuca rubra*.

Plate 1: Field site at Sourhope, 2000-2001

The experimental site at Newton Rigg (Plate 2) was representative of the type of inby land, with good quality grazing, that many farmers use for dip disposal if they do not have access to a suitable alternative. Application of dip was undertaken on two adjacent sites at Newton Rigg Experimental Farm (National Grid Ref.: NY364302), which is nine and a half miles West of Penrith in Cumbria. The sites at Newton Rigg represent fertile inby land, which is land that tends to be used more intensively and provides better quality pasture, is generally closer to farm buildings and is found on lower slopes in a valley than the rougher grazing land that is represented at Sourhope. Dip disposal at Newton Rigg was carried out on one site (Site A) in autumn 2001 and on two sites (Sites A and B) in spring

2002 using the same experimental design. The second Latin Square (Site B) was set up to allow comparisons of the effects of application on the new site that was only used once for dip disposal and the original site that was used in two consecutive years. The soil had an organic content of approximately 18.8%, a pH of approximately 4.4 and was situated at an altitude of approximately 225m above sea level. The vegetation consisted primarily of heavily grazed seeded rye grasses with isolated patches of *Juncus* sp., indicating areas of high soil moisture content.

Plate 2: Field site at Newton Rigg, 2001-2002

5.2.2 The Latin Square Design and Dip Application

At both Sourhope and Newton Rigg the Latin Squares were set up with five treatments and five replicates of each treatment (Figure 5.1); Cypermethrin at the recommended dilution for made up dip (SP), Cypermethrin, made up dip diluted 1:3 (SP dilute), Diazinon at the recommended dilution for made up dip (OP), Diazinon, made up dip diluted 1:3 (OP dilute) and control (water).

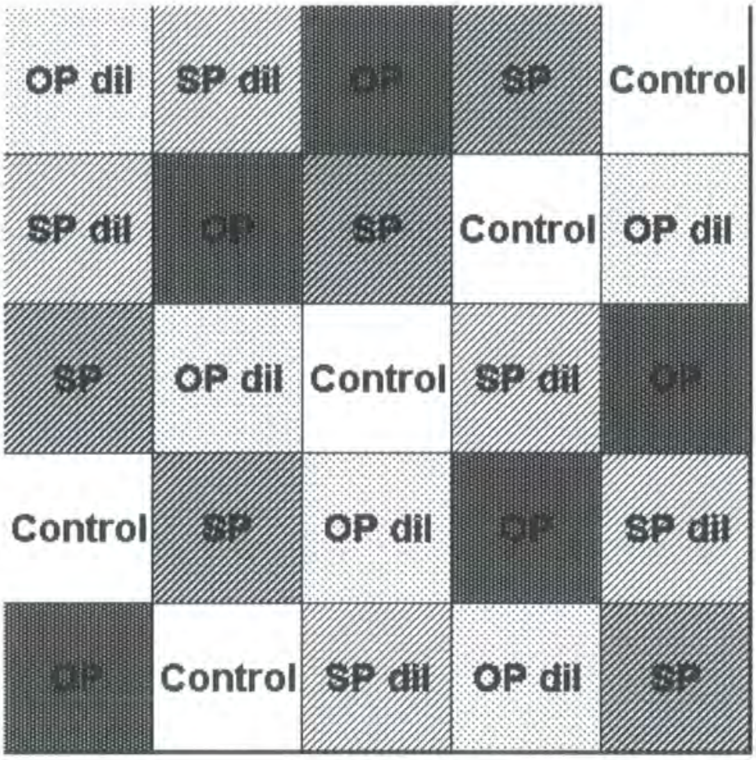


Figure 5.1: Latin Square Experimental set up at Sourhope and Newton Rigg Experimental Farms

The total plot size at Sourhope was 50m x 50m (constrained by available site dimensions), with individual plots therefore of 10m x 10m, and application rate was equivalent to $5000 l ha^{-1}$. The treatments were applied on 15 June 2000. Each treatment was spread to within 0.5m of the edge of each plot, effectively leaving a boundary of 1m between treatments.

Plot size for the Latin Squares at Sites A and B at Newton Rigg was 25m x 25m (constrained by available site dimensions), with individual plots therefore of 5m x 5m, and application rate was equivalent to 5000lha^{-1}. Each treatment was spread to within 0.25m of the edge of each plot, effectively leaving a boundary of 0.5m between treatments. In spring 2002 Site B was set up at Newton Rigg, adjacent to the Site A, to create additional data and allow between site comparisons. Site B was set up in exactly the same way as Site A but with the treatment plot arrangement turned by 90 degrees. The treatments were applied on Site A on 24/10/01 and to Sites A and B on 24/5/02.

The appropriate measure of dip for each plot was diluted on site with maximum ventilation and minimum possible exposure to participants and dip was applied using watering cans. Dip application was carried out according to safety guidelines (Health and Safety Executive, 1998), using recommended protective clothing and methods for storage of the dip prior to disposal.

5.2.3 Sourhope Sampling Regime

Soil sampling was carried out pre-disposal (15/06/00) and at 10 days (26/06/00), 20 days (04/07/00) and 40 days (24/07/00) after treatment application and in spring 2001 (16/5/01) (Table 5.1).

Sampling Technique	Predisposal Sampling	Time after sheep dip application			
		10 days	20 days	40 days	12 months
Soil Samples	✓	✓	✓	✓	✓
Pitfall Traps	×	✓	✓	✓	✓
Suction Samples	×	✓	×	✓	×

✓ indicates sampling was carried out
× indicates sampling was either not carried out or was unsuccessful

Table 5.1: The timing of each sampling technique used at Sourhope, 2000-2001

Soil sampling was carried out using the same method as used in the historic farm sampling (Chapter 3) but taking two smaller sample units ($12cm^3$) on each plot to reduce the possibility of taking single unrepresentative samples, such as those containing ant nests, that would provide misleading results at this scale. Mobile invertebrates were collected from each soil sample by heat extraction in Berlese funnels for one week.

Additional samples for the hand sorting of earthworms were not taken at Sourhope because earthworm densities were found to be too low to make this valuable.

A pitfall trap was placed at the centre of each plot. Each pitfall trap consisted of a plastic coffee cup, measuring 7 cm in diameter, containing approximately 50ml of ethylene glycol (Clark and Blom, 1992) and sunk level to the ground surface. These were in place prior to disposal but were destroyed by grazing sheep. The sheep were excluded before dip application and the pitfalls were replaced on the day the dip was applied. They were collected at 10, 20 and 40 days after the experimental treatment. A further 25 pitfalls were placed in similar positions, the following year (01/06/01). These were collected after 14 days (15/06/01). As in Chapters 3 and 4 the pitfall traps were not intended to provide density comparisons but to give comparative abundance estimates only since they capture invertebrates from an unknown area (Southwood and Henderson, 2000).

Suction sampling was carried out using the "Echo Blower-vacuum," with an extension sampling tube (aperture 0.01 m^2). Sampling was carried out for two 30s intervals within each plot (Macleod *et al.*, 1994) 10 and 40 days after treatment application. Suction sampling measures surface-active invertebrates, including invertebrates on vegetation, such as bugs, which spend little time on the ground and are therefore less likely to fall into pitfall traps.

Predisposal sampling on 15th June was considered too early in the season at such a northerly site for suction sampling to catch adequate numbers of surface-active invertebrates (i.e. adult insects) for meaningful analysis of results. Timing of suction sampling was also determined by the weather, as sampling on wet vegetation is not possible. Rainfall prevented suction sampling 20 days and 12 months post dip application.

5.2.4 Newton Rigg Sampling Regime

The soil sampling and invertebrate extraction methods at Newton Rigg were the same as those used at Sourhope (above). In 2001 soil sampling was carried out on Site A pre-disposal (11/10/01) and at 10 days (2/11/01) after treatment application. Further samples were not taken at 20 and 40 days after treatment application because many invertebrates overwinter in inactive stages and cannot be extracted (Table 5.2).

In 2002 soil sampling was carried out both on the original site (Site A) and the adjacent Site B predisposal (24/5/02) and at 10 days (3/6/02), 20 days (13/6/02) and 40 days (2/7/02) after treatment application.

A further soil sample of approximately 12cm^3 was taken from each plot for an investigation of densities of earthworms. These cores were sorted by hand on return to Durham.

Season of Application	Autumn		Spring				
	Time (in days) after sheep dip application						
Sampling Technique	**Predisposal Sampling**	**10**	Predisposal Sampling	**10**	**20**	**40**	**50**
Site A							
Soil Samples	✓	✓	✓	✓	✓	✓	×
Pitfall Traps	×	✓	×	×	×	×	×
Suction Samples	×	×	×	×	×	×	✓
Soil Cores for Earthworms	✓	✓	✓	✓	✓	✓	×
Site B							
Soil Samples	×	×	✓	✓	✓	✓	×
Pitfall Traps	×	×	×	×	×	×	×
Suction Samples	×	×	×	×	×	×	✓
Soil Cores for Earthworms	×	×	✓	✓	✓	✓	×
Soil Cores for Collembola	×	×	✓	✓	✓	✓	×

✓ indicates sampling was carried out
× indicates sampling was either not carried out or was unsuccessful

Table 5.2: The timing of each sampling technique used at Newton Rigg, 2001-2002

Pitfall traps were laid at Site A on 11/10/01, using the same method as at Sourhope, again to provide comparative abundance estimates, one near the centre of each plot. These were in place prior to disposal but were destroyed by grazing sheep. The sheep were excluded before dip application and the pitfalls were replaced on the day the dip was applied. They were collected 10 days after the experimental treatment.

Pitfall traps were not laid in 2002 as, following the 2001 results, the plot sizes of 5m x 5m were deemed too small to make this valuable. The active invertebrates, such as spiders and carabid beetles, that are captured in pitfall traps can cover large areas quickly and recolonisation on the small plots at Newton Rigg was likely to occur before the first post disposal sampling after 10 days. There was therefore no way to establish whether results that were not significant occurred because there was no effect of the sheep dip or because recolonisation was swift.

Suction sampling for surface-active invertebrates was undertaken only at 50 days using the same apparatus as at Sourhope. Heavy rainfall on the earlier sampling occasions made vegetation too wet to sample. Sampling was carried out for 6x10 second intervals within each plot (Macleod *et al.*, 1994) as this regime collected more invertebrates on the shorter grasses than the longer time intervals used on the longer grasses at Sourhope.

Additional sampling for Collembola

Collembola are very numerous and can occur at densities between 10,000 and 100,000 individuals per square metre in many terrestrial ecosystems (Hopkin, 2000). They can be sampled using several different methods and have been present in samples from Berlese extraction in other parts of this work in numbers far too great to count. More time efficient methods include suction sampling (Frampton, 2000, 2001), pitfall sampling (Holland *et al.*, 2002) and small soil cores for extraction of the invertebrates using Tullgren Funnels (Southwood and Henderson, 2000). The last method was chosen for this study as it overcame the need for the dry weather that is necessary for suction sampling and problems of disturbance that can occur with pitfalls.

Sampling for Collembola was carried out on Site B at Newton Rigg, set up in Spring 2002 predisposal (24/5/02) and at 10 days (3/6/02), 20 days (13/6/02) and 40 days (2/7/02) after treatment application. From each plot a $0.001m^2$ soil core was taken for extraction of the invertebrates in the laboratory using Tullgren Funnels (Brady, 1969). Extraction was into ethanol over a 48 hour period.

5.2.5 Identification of Invertebrates

The invertebrates collected were identified and sorted into major taxa according to Chinery (1993). Beetles were identified to species level by Dr J. Butterfield (Durham University) and spiders and bugs were identified to species level by Dr J. Woodward.

The invertebrates collected by soil sampling and heat extraction were divided for statistical analysis into active invertebrates, sedentary invertebrates and non-arthropods in the same way as in the farm study (Chapter 3). The different behaviour of the active and sedentary invertebrates might cause them to be exposed to the dip for different amounts of time and the speed of their reaction to the dip and recovery or recolonisation might also differ. Active invertebrates included; adult carabids, adult staphilinids, flies, ants, other Hymenoptera, spiders, harvestmen and centipedes. Sedentary invertebrates included; weevils, beetle larvae, tipulid larvae, other fly larvae, bugs, Lepidoptera and sawfly caterpillars. Non-arthropods included; earthworms, slugs and snails.

In the smaller soil cores taken for the Collembola study invertebrates were grouped as total invertebrates, Collembola and Acari.

The invertebrates collected in pitfall traps were sorted into major taxa and the totals from each treatment type were tested statistically. Elaterid and carabid beetles and linyphiid and lycosid spiders were then investigated separately as these taxa were numerous enough for individual statistical analysis.

The invertebrates captured by suction sampling were also sorted into major taxa. At Sourhope the bugs were identified to species level for separate statistical analysis because they were numerous and are important in the diet of newly hatched upland birds (Beintema *et al.*, 1991). At Newton Rigg bugs were not numerous but Collembola and Acari were recorded separately for comparison with the study of Collembola in the smaller soil cores.

5.2.6 Statistical analysis

Numbers of invertebrates were log-transformed and the results were analysed using ANOVA for Latin Squares. ANOVA is the most powerful and useful analysis for considering more than two groups in the randomised design (Dytham, 1999). Tukey HSD tests were carried out where the ANOVA results had been significant to isolate which treatment types were significantly different to each other.

5.3 Results of the experimental field trial at Sourhope

In the following section dip strength organophosphate and synthetic pyrethroid are referred to as OP and SP and the 1:3 diluted dips are referred to as OP dilute and SP dilute (OPd. and SPd. in Tables 5.3 and 5.4).

The results of the statistical analyses of the data collected at Sourhope are listed in Appendix 3. These tables include geometric mean numbers of invertebrates for each treatment, results of ANOVA tests and Tukey HSD tests where ANOVA revealed significant heterogeneity between the plots. Table 5.3 summarises the results showing the significant results of the ANOVAs and indicating which treatments were responsible for the significant reductions in densities of invertebrates on treated, compared to control, plots. All sampling methods indicated that some groups of invertebrates were at significantly lower densities on sheep dip disposal plots, compared to the control plots, at some time interval after application.

Sampling Method and Taxonomic Group	Time after sheep dip application			
	10 days	20 days	40 days	12 months
Soil Samples				
Total Invertebrates	NS	NS	NS	SP
Sedentary Invertebrates	OP	NS	NS	NS
Pitfall Samples				
Total Invertebrates	NS	SP	NS	NS
Lycosidae	NS	SP, SP dil.	SP	NS
Linyphiidae	NS	NS	NS	NS
Elateridae	OP, SP	SP	OP	NS
Carabidae	NS	NS	Control *	NS
Suction Samples				
Total invertebrates	OP, OP d., SP, SP d.	N/A	OP, OP d., SP, SP d.	N/A
Bugs	OP, OP d., SP, SP d.	N/A	OP, OP d., SP, SP d.	N/A
Hyledelphax elegantus	OP, OP d., SP, SP d.	N/A	(too scarce)	N/A
Pachytomella parallela	(too scarce)	N/A	OP, OP d., SP, SP d.	N/A

Key
N/A = not sampled NS = no significant differences * = significantly less on control

N.B. Where treatment abreviation is listed, eg OP, SP, OP d., SP d., significant reduction occured due to that treatment

Table 5.3: Summary of treatments responsible for significant reductions in densities of invertebrates on treated, compared to control plots, in the Latin Square experiment at Sourhope 2000-2001.

5.3.1 Soil fauna (soil samples)

There were no significant differences in total invertebrate densities on any of the sampling occasions in 2000. However, In the resample in spring 2001 the total invertebrate densities were significantly lower on the SP plots, whereas the SP (dilute), OP and OP (dilute) treated plots showed no significant difference from the control (Figure 5.2). Ten days after sheep dip application, the sedentary soil invertebrates were present at significantly lower densities in the plots where OP had been applied than in the controls, indicating an immediate effect (Table 5.3). At 20 days after application, the same pattern remained, with lower densities on the treated areas but the differences from the control areas were not statistically significant. At 40 days the pattern was no longer apparent.

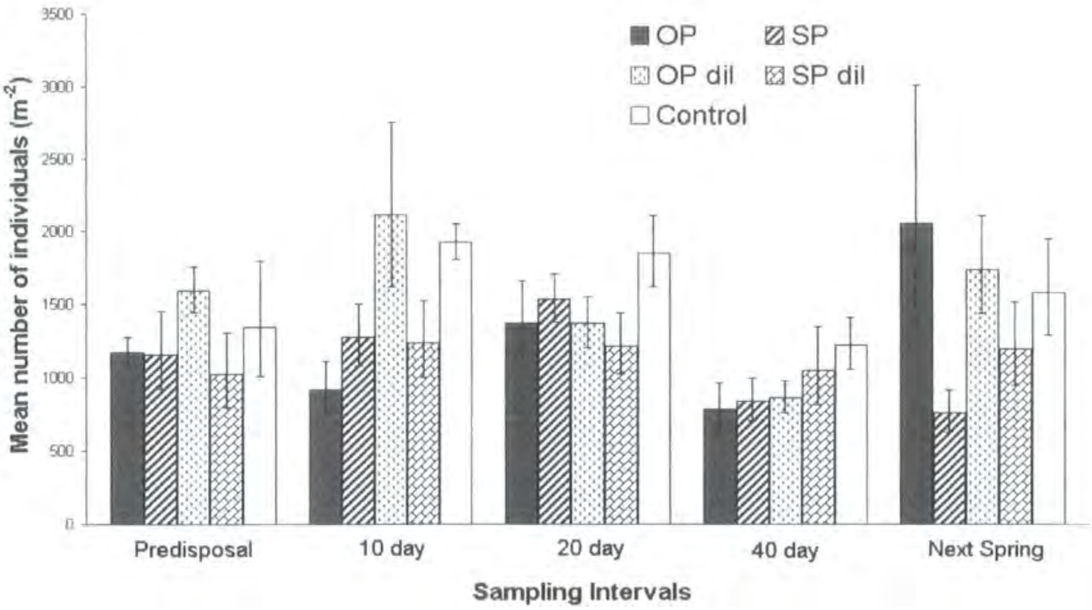


Figure 5.2: Geometric mean densities, with 95% confidence limits, of total invertebrates obtained by soil sampling at Sourhope before and at 10, 20 and 40 day intervals and 12 months after treatment application, 2000-2001

5.3.2 Surface invertebrates (pitfall traps)

The pitfall results depict a seasonal variation in the densities of surface-active invertebrates that is similar across the treatment types (Figure 5.3). However, differing effects of the treatments applied are present within the overall pattern. The pitfall results showed significant effects of dip disposal in the 20 day sample when significantly lower numbers of invertebrates per pitfall were caught on the SP plots, compared to the controls (Table 5.3, Figure 5.3).

Different taxa showed differing responses to the dip applications. Lycosid spiders were most affected by SP and were at significantly lower densities on both SP and SP (dilute) plots than on the controls 20 days after application and remained significantly lower on the SP (undiluted) plots after 40 days (Table 5.3). Elaterid beetles, were significantly adversely affected by the undiluted OPs and undiluted SPs 10 days after application. Samples at 20 days showed significantly lower numbers for SP plots and at 40 days there were significantly lower numbers on OP plots. Carabid beetles showed no statistically significant differences in densities between control and treated plots until 40 days after dip application and then numbers were significantly higher on the SP (dilute) plots than on the controls. There were no significant differences between treated and control plots in the pitfall catches in the spring of 2001, the following year.

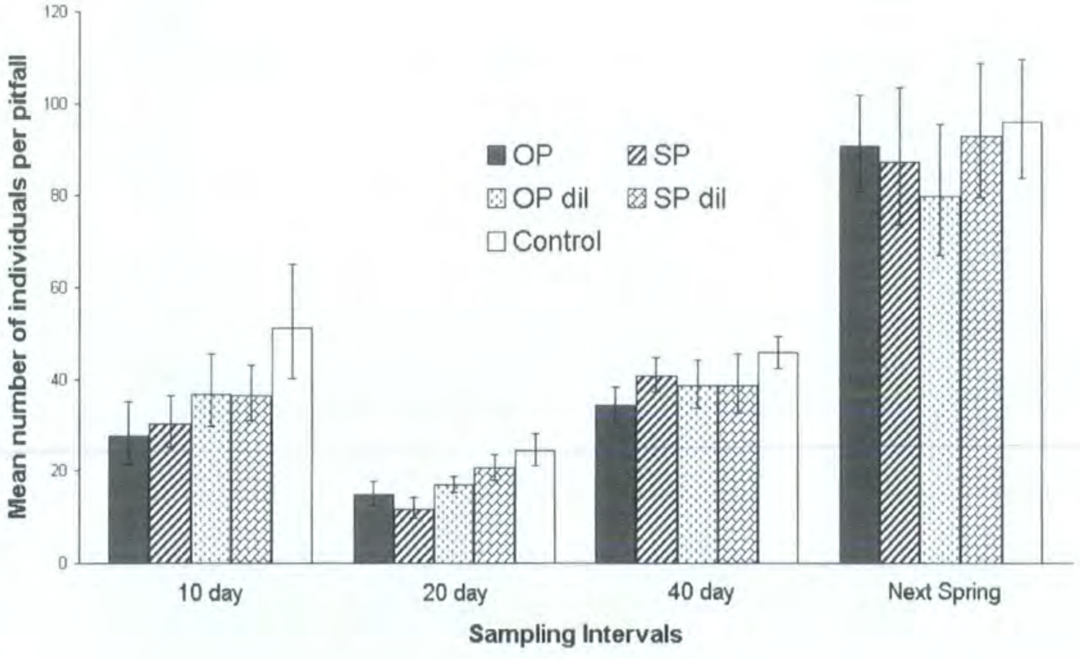


Figure 5.3: Geometric mean numbers, with 95% confidence limits, of invertebrates obtained by pitfall trapping at Sourhope before and at 10, 20 and 40 day intervals and 12 months after treatment application, 2000-2001

5.3.3 Surface invertebrates (suction samples)

The suction samples indicated that invertebrates at the soil surface and on the vegetation were severely depleted after dip application, with the diluted dip acting as adversely as the undiluted (Table 5.3). Ten days after dip application the numbers on the disposal plots were approximately 20% of those on the control plots. There was little recovery by 40 days and at both 10 and 40 days there were significantly lower numbers on all of the dip treatments compared with the controls (Figure 5.4).

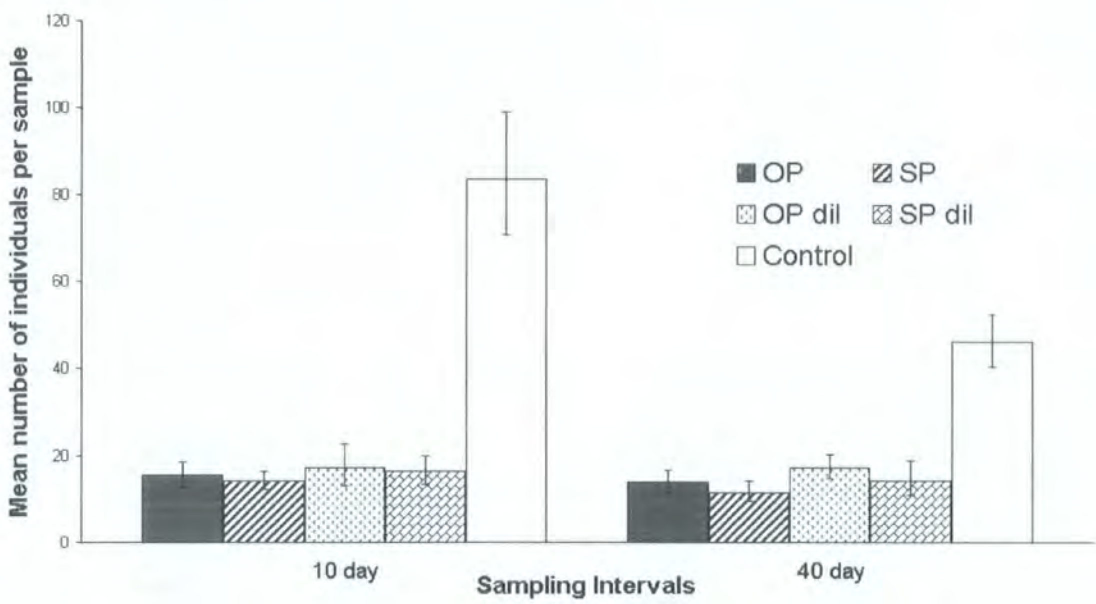


Figure 5.4: Geometric mean numbers, with 95% confidence limits, of invertebrates obtained by suction sampling at Sourhope at 10 and 40 day intervals after treatment application, 2000

The bugs (Hemiptera) comprised more than 60% of the invertebrates taken in the suction samples and they showed the same trend as the total surface invertebrates with significantly lower numbers on all the disposal treatments at both 10 and 40 days. Two Hemiptera species were taken in sufficiently high numbers to analyse the influence of dip disposal at the species level. At 10 days *Hyledelphax elegantulus* was present at significantly lower densities on all dip disposal treatments compared with the controls. At 40 days *H. elegantulus* numbers had declined over the whole of the experimental area and it had been replaced by *Pachytomella parallela* which was at significantly lower densities on all the disposal areas compared to the controls.

5.4 Results of the experimental field trial at Newton Rigg

A summary of treatments responsible for reductions in densities of invertebrates on treated, compared to control plots for all sampling methods in 2001 and 2002 at Newton Rigg is shown in Table 5.4. The results of the statistical analyses including geometric mean numbers of invertebrates for each treatment are given in Appendix 3 with results of ANOVA tests and Tukey HSD tests where ANOVA revealed significant heterogeneity between the plots.

Table 5.4: Summary of treatments reponsible for significant reduction in densities of invertebrates on treated, compared to control plots, in the Latin Square experiments at Newton Rigg

Sampling Method and Taxonomic Group	Autumn Application	Spring Application			
		Time after sheep dip application			
	10 days	10 days	20 days	40 days	50 days
Site A					
Soil Samples					
Total invertebrates	OP, SP	NS	OP, SP, SP d.	NS	N/A
Sedentary invertebrates	OP, SP	NS	OP, SP, OP d., SP d.	SP	N/A
Active invertebrates	OP, SP, OP d.	NS	NS	NS	N/A
Earthworms	NS	NS	NS	*	
Pitfall Samples					
Total Invertebrates	NS	N/A	N/A	N/A	N/A
Suction Samples					
Totals	N/A	N/A	N/A	N/A	NS
Collembola	N/A	N/A	N/A	N/A	NS
Mites	N/A	N/A	N/A	N/A	OP, SP, OP d., SP d.
Site B					
Soil Samples					
Total invertebrates	N/A	NS	OP, SP, OP d., SP d.	NS	N/A
Sedentary invertebrates	N/A	OP, SP, OP d., SP d.	OP, SP, OP d., SP d.	NS	N/A
Active invertebrates	N/A	NS	NS	NS	N/A
Active invertebrates A+B	N/A	NS	OP, SP, SP d.	NS	
Earthworms	N/A	NS	NS	NS	N/A
Suction Samples					
Totals	N/A	N/A	N/A	N/A	NS
Collembola	N/A	N/A	N/A	N/A	NS
Mites	N/A	N/A	N/A	N/A	NS

Key
N/A = not sampled * = significantly less on control NS = No significant differences

N.B. Where treatment abreviation is listed, eg OP, SP, OP d., SP d., significant reduction occured due to that treatment

5.4.1 Autumn Sampling 2001

5.4.1.1 Soil fauna (soil samples)

Predisposal samples showed no significant differences in invertebrate densities between plots used in the treatment regime. Ten days after sheep dip application, the total soil invertebrates were present at significantly lower densities in the plots where OP (42% lower than the controls) and SP (24% lower than the controls) had been applied, indicating an immediate effect (Figure 5.5). This pattern was repeated in both sedentary invertebrates and active invertebrates although the latter also showed significantly lower densities on plots where diluted OP had been applied. The samples hand sorted for earthworms showed no significant differences between the plots before or after treatment application.

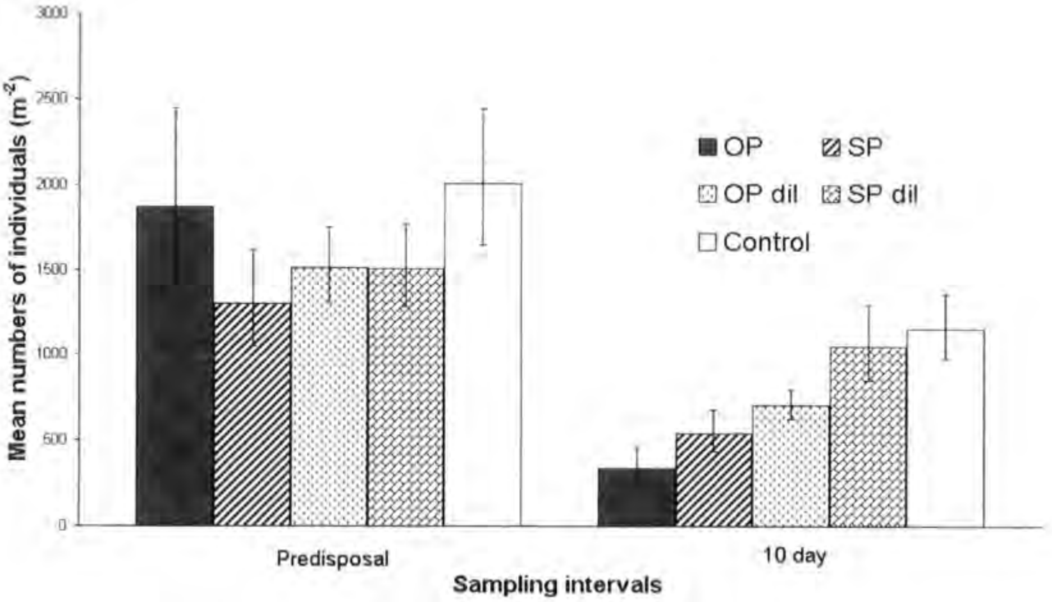


Figure 5.5: Geometric mean densities, with 95% confidence limits, of invertebrates obtained by soil sampling in Site A at Newton Rigg predisposal and 10 days after treatment application in autumn 2001

5.4.1.2 Surface invertebrates (pitfall traps)

The pitfall results did not show a significant reduction of total invertebrate activity 10 days after dip disposal and invertebrate numbers were low on all plots (Appendix 3).

5.4.2 Spring Sampling 2002

5.4.2.1 Soil fauna (soil samples)

Predisposal

Predisposal samples showed no significant differences for total, sedentary or active invertebrates either at Site A, which had been treated in the previous autumn, or Site B which had not previously been exposed to sheep dip.

Total Invertebrates

On Site A there were no significant differences in total invertebrates 10 days after dip application but by 20 days total invertebrate densities were significantly lower on plots treated with SP, OP and diluted SP compared with the control. By 40 days there were no significant differences (Figure 5.6).

On Site B there were no significant differences in total invertebrates at 10 days but by 20 days SP, OP, diluted SP and dilute OP showed significantly reduced numbers of total invertebrates. By 40 days there were no significant differences (Figure 5.7).

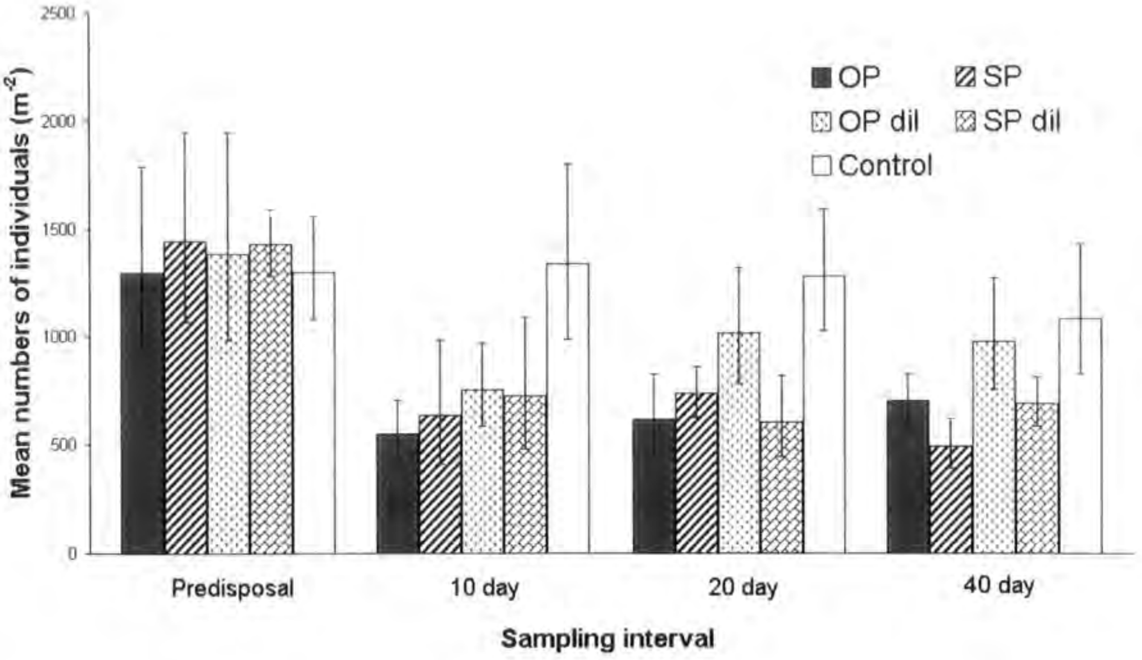


Figure 5.6: Geometric mean densities, with 95% confidence limits, of total invertebrates obtained by soil sampling at in Site A at Newton Rigg predisposal and at 10, 20 and 40 day intervals after treatment application, 2002

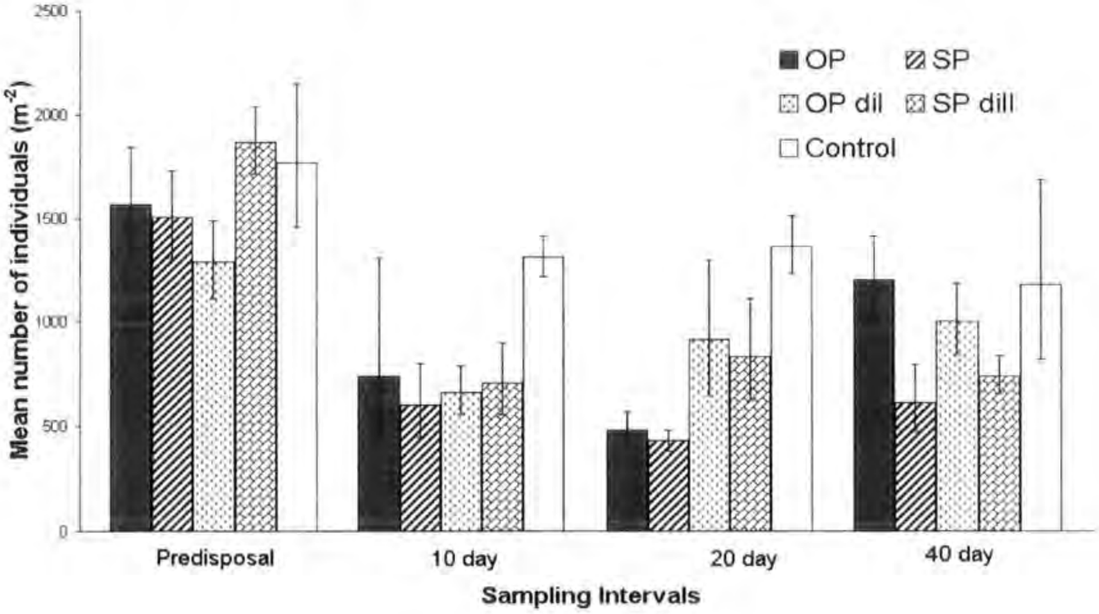


Figure 5.7: Geometric mean densities, with 95% confidence limits, of total invertebrates obtained by soil sampling at in Site B at Newton Rigg predisposal and at 10, 20 and 40 day intervals after treatment application, 2002

Sedentary Invertebrates

At Site A there were no significant differences in sedentary invertebrates at 10 days but at 20 days there were significant reductions on all the treated plots compared to the controls. By 40 days there were still significant differences between the undiluted SP plots but not between the diluted SP or either of the OP applications when compared with the controls.

At Site B significant differences in sedentary invertebrates were found at 10 days when all the treated sites were compared with the control and at 20 days this pattern remained. By 40 days there were no significant differences between the treated and control plots for sedentary soil invertebrates.

Active Invertebrates

When tested independently of each other there were no significant differences in active invertebrates between the treated and control plots on any sampling occasion at either Site A or Site B. However, it was noted that the geometric means were far greater on the control plots post disposal than on any of the treated sites. This did not reveal statistically significant results due to the patchy nature of active invertebrate activity resulting in non-homogeneity of the variances of the samples and high standard errors. ANOVA assumes the samples have equal variances (Dytham, 1999) and although the variances in this data are not significantly different they are responsible for the false negative result. Adding together the results from both sites, which were very similar, revealed significantly reduced densities of active invertebrates at the 20 day sampling occasion on SP, OP and dilute SP treated plots compared with the control (Appendix 3).

Earthworms

At Site A there were no significant differences between the densities of earthworms on treated and control plots after 10 and 20 days. At 40 days significantly higher densities were found on SP, OP and dilute SP treated sites when compared with the controls. There were no significant differences in earthworm densities on any sampling occasion at Site B.

5.4.2.2 Surface invertebrates (suction samples)

At 50 days there were no significant differences in total invertebrate densities between the treated plots and the control at either Site A or Site B. There were also no significant differences in Collembola at either site. However, at Site A there were significantly decreased numbers of mites on SP, OP, dilute OP and dilute SP plots when compared with the control. There were no significant differences in mite densities at site B. Bugs and spiders were not caught in sufficient numbers to make statistical analyses of separate species valuable.

5.4.2.3 Collembola Soil Samples

Results of the Anova and Tukey HSD tests are in Appendix 3.

There were no significant differences between numbers of total invertebrates on the treated and control plots on any of the sampling occasions. However, at 20 days the number of Collembola was significantly greater on the control site than on the sites treated with OP, SP and dilute OP (Figure 5.8). There were no significant differences by 40 days.

10 days after dip application there were significantly fewer mites on the OP dilute treated area (53% less) compared with the control. 20 days after treatment application there were still significantly greater numbers of mites on the control area compared with the OP

dilute treated area (66% less) and also with the undiluted SP treatment (36% less). By 40 days there were no significant differences between any of the treated plots and the control.

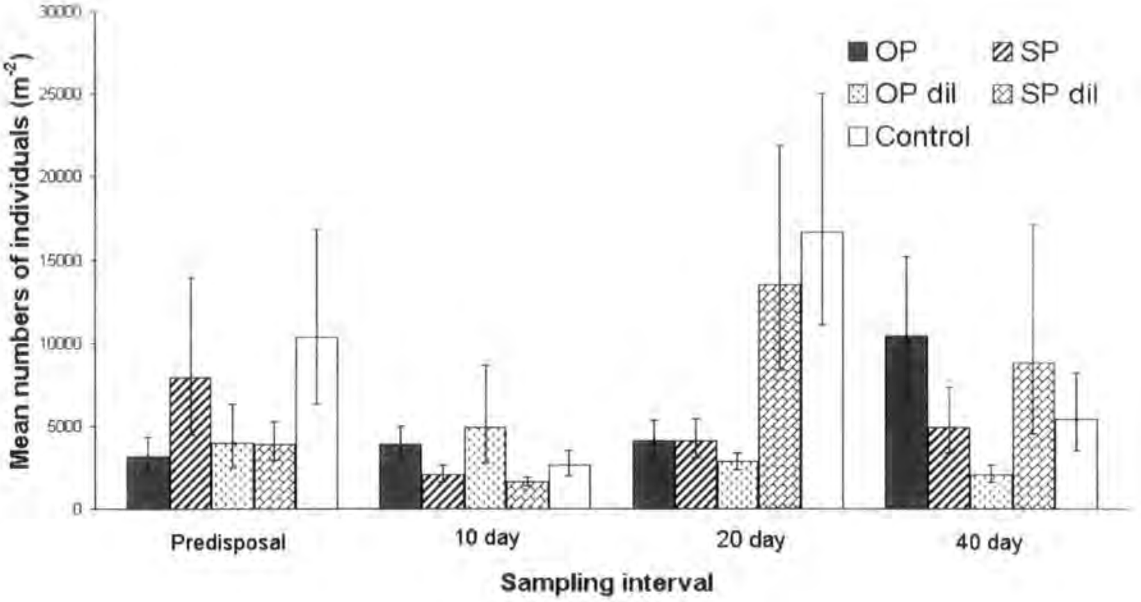


Figure 5.8: Geometric mean numbers, with 95% confidence limits, of Collembola obtained by soil sampling at in Site B at Newton Rigg predisposal and at 10, 20 and 40 day intervals after treatment application, 2002

5.5 Discussion

All sampling methods used in the Latin Square experiment at Sourhope indicated significant adverse effects of spring dip disposal on invertebrates in rough pasture. Sampling at Newton Rigg on fertile inby land also indicated a detrimental effect of sheep dip disposal on terrestrial invertebrates in both spring and autumn. The proportions of invertebrates in each taxon captured at Newton Rigg (Plate 2) differed from those on the rougher grazing land at Sourhope (Plate 1). In particular there were fewer bugs at Newton Rigg, possibly due to the short grazed grass providing less suitable habitat for many species. There was also a greater proportion of flies at Newton Rigg, probably due to the increased amount of manure in the more intensively grazed field.

At Newton Rigg there were differences in intensity and duration of the effects of the different dips and dilutions on different invertebrate taxa. For example, in spring there were significantly lower sedentary invertebrate densities on all treated plots compared to the controls on Site A after 20 days but by 40 days only the SP treated plot had significantly lower densities. At Site B significantly lower sedentary invertebrate densities were found on treated plots after 10 days, whereas these effects were only apparent on total invertebrates after 20 days (Table 5.2). The effects on active invertebrates were significant after 20 days when the results from both Site A and Site B were combined. The ANOVA single site results did not show significant results individually due to the large inter-sample variability. The active invertebrates were comprised mainly of flies, which lay their eggs in clusters, causing group emergence. This phenomenon was seen predominantly in the control plots, probably due to adults selecting breeding grounds without dip or the dip affecting the eggs/larvae, as seen in the sedentary invertebrate results.

The different responses between the invertebrate taxa are probably due not only to differing susceptibilities (Jepson, 1989) but also to differences in exposure to the dip. For instance, sedentary invertebrates would not be expected to be able to escape an area affected by pesticides as fast as active invertebrates. In addition, the ability of the invertebrates to withdraw to deeper levels of the soil profile may determine the level of exposure to a pesticide (Wallwork, 1976). The suction samples at Sourhope consisted largely of arthropods from the vegetation. These would have been directly exposed to the dip and it was not surprising that suction sampling indicated the most severe effects, with major reductions in densities for all insecticide treatments. These findings are also reflected in an experimental study in which a small carabid *Bembidion lampros* was caged on mature wheat leaves and at the soil surface 24 h after insecticide application (Cilgi *et al.*, 1988). Much higher mortality occurred on the wheat leaves. The behaviour of invertebrates and their chosen habitat within the disposal area is therefore important in determining their response to the presence of a pesticide.

In the present study the contribution of the persistence of dip to the lack of recovery on the treated areas could not be estimated. The majority of arthropods caught by suction sampling were bugs. As these are relatively immobile and many have annual life cycles, recovery after a single, short-lasting lethal event would not be expected within the year. *Pachytomella parallela* adults, which showed reduced densities on the treated areas after 40 days, were most unlikely to have moved onto the plots after the dip application. *P. parallela* was the only mirid adult to be caught at 40 days and unidentified mirid nymphs were significantly depleted on the disposal plots at 10 days. As nymphs, bugs tend to remain on the same plant and even small areas would not be recolonised within a season. The reduction in Hemiptera densities after disposal may be of particular interest since bugs are important chick food for upland waders such as lapwing and redshank (Beintema *et al.*, 1991).

Soil sampling at Sourhope suggested the possibility that effects of SP dip persisted for up to 12 months. In the spring of 2000, although reductions in invertebrate densities occurred on all treated plots, the single significant reduction was in the sedentary invertebrates on the OP plots 10 days after application (Table 5.3). In Spring 2001, however, densities on the SP plots were significantly lower than on the control areas. It is unlikely that this is a statistical anomaly because both SP and SP dilute treatments show depressed numbers whereas densities on the OP plots are both similar to the controls. Any degree of long-term persistence in Cypermethrin has potentially serious consequences for terrestrial invertebrates, as it is one of the least selective insecticides (Thieling and Croft, 1989). Roberts and Standen (1977;1981) revealed that Cypermethrin has half lives in different soils ranging from 1 to 10 weeks but unextractable residues were still found up to 52 weeks after the Cypermethrin was introduced to the soil. If all the dip residue is not removed by the time of the next disposal effects could be expected to be stronger and/or last longer than a single disposal. Such effects may build up over time and produce long-term decreases in the invertebrate populations, such as at the repeatedly used disposal sites of Derwent reservoir, Teesdale 1 and Yorkshire 4 (Chapter 3).

The re-sample at Site A at Newton Rigg in the spring, prior to re-disposal, showed no significant effects from dip disposal in the previous autumn. However, at 20 days after the spring disposal there were significantly fewer sedentary invertebrates on all of the treated plots when compared to the controls but by 40 days recovery had occurred on all plot types apart from the undiluted SP. Additionally, 50 days after treatment application at

Newton Rigg, despite repeated heavy rainfall post disposal, there remained a significant effect on numbers of mites on all treated plots obtained by suction sampling at Site A, but not at Site B, which might indicate a cumulative effect of the repeated dip disposal.

The pitfall catches at Sourhope indicated a drop in total numbers on the SP plots at 20 days and lycosid spiders, in particular, were susceptible to SP and not OP. These results agree with other studies that suggest that synthetic pyrethroids are particularly toxic to spiders (Frampton, 2001; Wiles and Jepson, 1992; Pullen *et al.*, 1992). At 40 days, total numbers did not differ significantly from the controls. This is consistent with a lack of persistence of either insecticide in the habitat of the surface active invertebrates but it could also be explained by the pitfall catches consisting predominantly of active predatory arthropods. These run rapidly over the soil surface, covering considerable distances within a short time (Thiele, 1977). Individuals running into the treated plots from outside would therefore have had little time to be adversely affected. In addition the vegetation coverage, in this type of disposal area with long grasses, may have afforded a considerable degree of protection to soil surface species, at the time of dip application. Adult elaterid beetles showed longer-term effects and this may reflect their association with the vegetation as well as their susceptibility (Cypermethrin is used to control *Agriotes* spp.) (Jepson, 1989). Unlike any of the other groups, the ground beetles were caught in significantly higher numbers on treatment plot (SP dilute) after 40 days. This could be a statistical anomaly but invasive species are likely to colonise pasture following insecticide application (Rushton *et al.*, 1989). In studies of the effects of DDT treatments carabid species varied in their response but *Nebria brevicollis* and *Trechus quadristriatus* showed increases after spraying, which was attributed to a rise in prey abundance following a pesticide related decline in other predator species (Wallwork, 1976).

Collembola densities were investigated, using the additional small soil cores, only on Site B at Newton Rigg, which had not been treated with dip prior to the spring, so the possibilities of cumulative effects of dip disposal were not investigated in this part of the study. Both OP and SP based dips adversely affected Collembola 20 days after dip application but effects were not significant after 40 days. Unfortunately there were too few carabids caught at this site throughout the main investigation to be able to provide any links between Collembola and carabid activity. However, adverse effects on the Collembola could mean carabids such as *Agonum dorsale* that specialise on Collembola may have to look to other sources of food on dip disposal sites or move to other areas (Burn, 1989). However, a change in ratio or quality of different prey is more likely than an absolute shortage of alternative prey items (Burn, 1989). The resultant diet may be sub optimal, as found for carabids in the areas of highest pesticide use in the Boxworth study where Collembola were significantly reduced (Frampton, 2001). Studies by Frampton (1997, 2000, 2001) and Holland *et al.* (2002) showed long term deleterious effects of pesticide regimes on Collembola when repeated over seven years. Since the regulations following the move to dispose of dip onto farmland limit farmers to specific areas for disposal, it is realistic to suppose certain areas will be used repeatedly over many years and this could have long term effects both on the Collembola populations and on the larger invertebrates that feed on them.

Mites were also found to be adversely affected by the disposal of sheep dip using the small soil cores at 10 and 20 days but showed recovery by 40 days. However, suction sampling on Site A at Newton Rigg 50 days after dip application showed significant reductions in mite densities on all treated sites compared to the controls. Mites play a role in the decomposition and recycling of organic material (Pechenik, 1996) and the role of mites in predator prey interactions can be dramatically altered when mite densities are lowered by pesticide application (Edwards *et al.*, 1967). Where recovery is slow due to a restricted breeding season or long life cycle as in cryptostigmatid mites (Wallwork, 1976) declines in density will persist for longer periods. If effects persist after repeated dip disposal, the essential decomposers and smaller organisms within the soil may be reduced. Suppression of microbial respiration and reduced nitrification in soil has been a noted side effect of several pesticides including Dieldrin and DDT and this has possible repercussions for soil processes in the long term (Wallwork, 1976). Reductions in decomposers and small soil organisms could then play a more major role in affecting the larger invertebrates, by altering their habitat and removing some small prey items.

The Latin Square Experiments at Sourhope and Newton Rigg both showed significant depletions of invertebrate populations post dip disposal followed by recovery in both surface active invertebrates from pitfall catches and soil invertebrates by 40 days. Although it might be expected that recolonisation would be more rapid on the smaller plots used at Newton Rigg there was no difference in recolonisation apparent between Sourhope and Newton Rigg during this study. However, at field scale (Chapter 3) multivariate analysis indicated that carabids, at least, were affected up to six months after dip application. The bugs at Sourhope did not recover or recolonise after exposure to dip because they are relatively immobile and have a yearly life cycle. Active invertebrates such as adult beetles and spiders often have a longer life cycle and can move over the surface faster than sedentary invertebrates such as beetle and fly larvae can move through the soil. Active populations would therefore be expected to recolonise more efficiently than sedentary invertebrates at a field scale. However, by 40 days the sedentary invertebrate population is likely to have been increased by active invertebrates such as adult diptera flying on to a disposal site some time after disposal but unable to detect the dip. They lay eggs that could hatch successfully if enough time had elapsed post disposal and by 40 days would be counted as larvae. It is therefore difficult to determine from the Latin Square investigations whether recovery or recolonisation is responsible at this scale.

Both closer cropped inby land and areas of rougher grazing land are used by wading birds for nesting and feeding (Appendix 4). The experiments detailed in this work have shown affects on a variety of land-use types and a variety of different invertebrates that are either prey items for wading birds or often prey items for the larger invertebrates, which are then taken by the birds. The apparent adverse effects of sheep dip on mites also indicates a possibility of disruption of important soil processes in disposal areas that, if allowed to persist by repeated dip disposal, could alter the soil fauna over a number of years.

6. ASSESSMENT AND LIMITATION OF THE RISK OF DIP DISPOSAL TO UPLAND BIRDS

The investigation into the effects of sheep dip disposal onto farmland has highlighted the potential risks to terrestrial invertebrates and upland birds. In an attempt to allow some approximate preliminary quantification of the risks a basic risk assessment model is proposed based on data gathered in this study and data from other relevant studies on terrestrial invertebrates and upland birds. Complete data are not currently available about the dietary requirements and movement patterns of each bird species at different ages and invertebrate community composition and densities vary according to location. The model therefore groups as much available data as possible together to provide a very approximate assessment of risk to young wading birds in an upland environment. Specific data about each bird species and each area would need to be input to make this model anything other than indicative but the proposed model is intended to provide a framework for future risk assessment as more data become available.

The birds most relevant to this study are Curlew, Lapwing, Redshank, Snipe and Golden Plover as these are the wading birds that many upland SSSIs are designed to protect and would most likely be affected by the dip disposal process. A thorough risk assessment must include both short and long term risks, direct and indirect effects of dip disposal onto farmland and take into account the very variable nature of current dip disposal practice as highlighted in the farm questionnaires (Chapter 2). The following risk assessment is intended to assess the possible consequences of a decline in invertebrate availability for upland wading birds. However, it does not include direct toxic effects of the dip to birds as there is no data currently available on this to input into the model. The basic estimates of risk are intended to be applicable over any area and subsequently used in conjunction with any specific relevant information available about the area.

Adult birds can travel over great distances to feed and are therefore unlikely to be affected by a drop in invertebrate prey on the relatively small areas used for sheep dip disposal. Chicks, however, are more restricted in their movement and lapwing chicks have been reported to stay around the nest until the entire brood has hatched and often for the first day (Cramp, 1983). Chicks are therefore more likely to be affected by areas of depletion in prey availability and are the main focus for this risk assessment.

Detailed quantitative information about wading bird diets was sparse for chick diets compared to adult birds. However, it is generally accepted that chick diets are very similar to the adult diets, restricted only by the shorter bill length in the very young birds which allows for shallower probing in search of soil invertebrates. For example, young Lapwing chicks feed almost exclusively on surface active invertebrates, particularly carabid beetles (Baines, 1990). Birds are opportunistic feeders and capitalise on the most abundant or accessible suitable prey in an area (Cramp, 1983). A calculation of the overall biomass required is therefore the most important factor in terms of bird feeding requirements.

The proposed risk assessment comprises the following steps:

- Calculate the reduction in biomass of potential invertebrate prey items from experimental disposal areas when compared with controls
- Determine the size of disposal area (where reduction in invertebrate biomass has occurred as a consequence of dip disposal), which would adversely affect the wading birds as either chicks or adults.

6.1 Methods for proposed risk assessment model

6.1.1 Invertebrate bird food requirements

Information about the different invertebrate taxa that comprise the bird's diets for both adults and chicks was obtained by reviewing available literature (Appendix 5).

6.1.2 Change in available biomass following sheep dip disposal

The invertebrate samples from rough grazing land at the experimental farm site at Sourhope in 2000 (Chapter 5) were used as a basis for the calculation of the overall change in available invertebrate biomass following sheep dip disposal. The invertebrates that had been extracted from all soil samples from predisposal (15/06/00) and 10 days (26/06/00), 20 days (04/07/00) and 40 days (24/07/00) and suction sampling at 10 and 40 days after disposal were dried to a constant weight at $70^{o}C$. Dried samples were weighed using a balance accurate to one thousandth of a gram. An arithmetic mean of the results from each treatment type was calculated for each sampling occasion. The percentage change in biomass between predisposal sampling and 40 day sampling was calculated for each treatment type. Undiluted OP treated plots had decreased in available invertebrate biomass by 63%, undiluted SP had increased by 6%, dilute OP had increased by 6% and dilute SP had decreased by 12% between predisposal and 40 days. The biomass on the control plots increased by an average of 143%, which would be expected on untreated plots during this time. These data were then used in a calculation to determine the number of chicks treated areas can sustain.

An average of the predisposal biomass ($0.9gm^{-2}$) based on the invertebrate standing crop was used as a starting point because there were no possible effects of dip disposal at this point. This was converted into energy using a conversion rate of 1g biomass to 25 KJ of usable energy to give initial energy (K). This conversion was an average of the KJ value per g of several important invertebrate species including tipulid larvae, ants, Coleoptera spp. and Diptera spp.

The total energy available following the application of each different treatment type is KX, where X is the estimated change in biomass (X_{SP}, X_{OP}, X_{SPdil}, X_{OPdil}, $X_{control}$). KX values were calculated per hectare and represent a standing crop. A factor F indicates the percentage of invertebrates expected to be available to the chicks, i.e. on or close to the soil surface or vegetation. This was determined using the results of the soil and suction sampling at Sourhope in Phase 1, which were the only complete sets available at the time of calculation. The proportion of surface invertebrates was calculated to be 44% of the total available invertebrates. Therefore we assume that KXF is the total energy from invertebrate material available to the chicks.

6.1.3 Distances travelled by broods to fulfil energy requirements

Detailed information about chick movement and energy requirements is incomplete for all the relevant bird species. However, the information for all the species was amalgamated to create an effective overall estimation.

Work on curlew chicks (Grant, unpublished) found that for broods studied to at least 22 days of age the mean maximum distances broods were recorded from their nests was approximately 197m. A similar figure was put forward by Cramp (1983) who suggested curlew chicks usually remain within 200m of the nest until fledging at four to five weeks. Lapwing chicks may move between 50 and 150m from the nest after the first week and up to 250m in the second week, but they have been found to remain close to the nest in the early days (Cramp, 1983). Smaller, younger chicks need to return to the nest more often and can move shorter distances than older chicks, which may not need to return to the nest at all. A linear relationship between distance travelled and time was assumed as a suitable means of breaking this information down. The distance of 197m (giving a maximum area coverable of approximately 120,000m^2) was therefore divided by 22 days to give a daily increase in possible distance moved from the nest. The maximum area within the range of the daily possible distance travelled was then calculated. It is assumed that the entire range over which the chicks can move is affected by sheep dip disposal to the same degree and they are not feeding on untreated land at any time.

6.1.4 Realistic foraging area within the range of the chicks

Chicks do not realistically feed over the entire area within the potential range, making repeated forays from the nest in search of food rather than searching over the whole area. Whittingham *et al.* (2000) found that breeding golden plover foraged in only 17 out of 85 fields in the study area. Radio-tracking of 22 broods of golden plover (Whittingham *et al.*, 2001) revealed an average of 0.157% of the potential home range was used for foraging (Whittingham, unpublished data, Appendix 6). KXF was multiplied by the calculated usable area in hectares (A) by the chicks to give a value of the estimated possible energy from invertebrate prey items available to the chicks of varying age and mass.

6.1.5 Calculation of daily energy requirements of the chicks

The daily energy requirements of chicks were calculated based on work by Schekkerman and Visser (2001) on Lapwing chicks. The daily metabolised energy is proportional to the size of the chick and is calculated using the following equation:

$$ME = 4.365 \times M^{0.911}$$

where ME is Metabolised Energy and M is the mass of the chick

Assuming that the chick has an approximate starting mass of 20g upon hatching (Schekkerman and Visser, 2001 for Lapwing; Pearce-Higgins and Yalden, 2002 for Golden Plover), a mean increase of 5g per day was added using the average daily growth rate of lapwing chicks from Schekkerman and Visser (2001) and assuming a linear relationship, which has been found to occur between at least five and thirty days (Baines, 1990). Lapwing chicks fledge at 70-80% of adult mass (Beintema and Visser, 1989a). The value of 202g for adult mass was taken from Schekkerman and Visser (2001) giving an 80% fledging mass of 160g, after which point it can be assumed that the chicks are highly mobile and can move out of dip disposal areas for more abundant invertebrate supplies if necessary.

6.1.6 Extrapolation of calorific requirements of chicks in relation to availability on disposal areas

By dividing the possible available energy from the invertebrate food source by the metabolised energy a value for the number of chicks that can be supported with increasing age, mass of chick and mobility.

The complete calculation is therefore:

$$\frac{KXFA}{ME} = N$$

Where:

N is the number of chicks of a certain age and mass that can be supported on disposal land.

KXF is the total energy from invertebrate material available to the chicks.

A is the area the chick could use for feeding

ME is metabolised energy requirements of each chick

Using this equation, given a starting biomass and depletion rate for dip type applied, it is possible to calculate either the critical disposal area that may cause problems for young chicks or the size of chick that would cope with depletion give proposed affected area (see case studies).

A usual clutch size for Lapwing chicks is 3 to 4 (Baines, 1988). Therefore, for the above calculation, if the area was found to be able to support less than 3 chicks it can be assumed that chicks would not have the optimum invertebrate food intake and fitness may suffer.

This model does not take into account any improved efficiency of feeding that may occur with age e.g. bill length increase, which allows probing for soil invertebrates at greater depths.

6.1.7 Summary of Assumptions

The above model uses the following assumptions:

- The nest, containing a clutch size of 3 to 4 chicks, is situated in a large disposal area. The chicks cannot move far enough at any stage prior to fledging to forage for invertebrates on untreated land.
- Total energy available is calculated using invertebrate data from the treated plots on rough grazing land at Sourhope and represents a standing crop, with the biomass remaining static over time. Total energy available might also be expected to differ depending on quality and type of land but this is not currently featured into the model. Different data, where available, could be input into the model for specific case studies.
- A linear relationship is assumed between age of chick and distance it can travel each day (Figure 6.1).

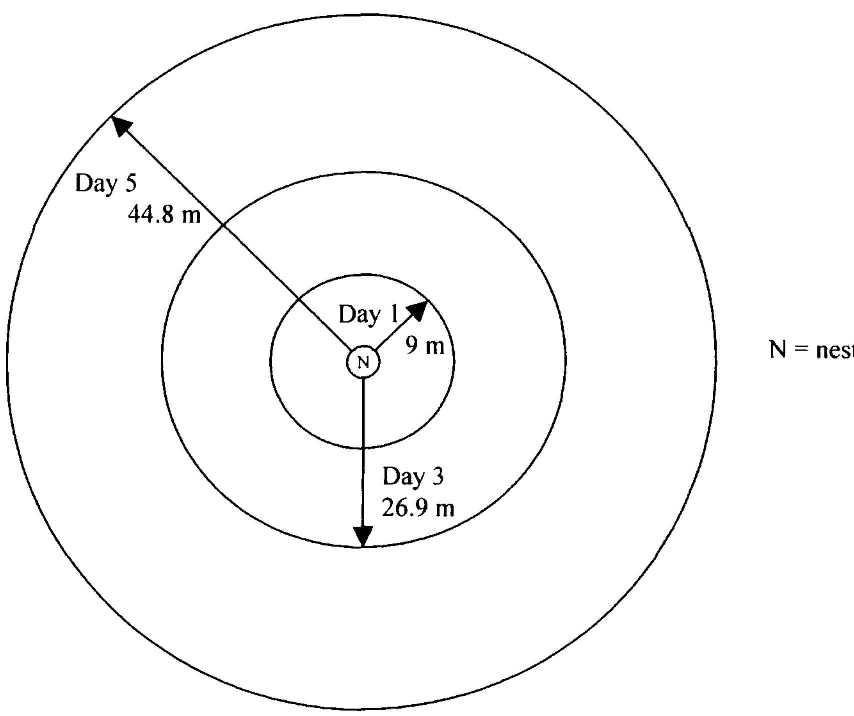


Figure 6.1: A representation of the increasing home range of chicks in the first five days after hatching

- 0.157% of the potential home range is used by chicks for foraging (Whittingham, unpublished, Appendix 6)
- Each chick has an approximate starting mass of 20g upon hatching and gains 5g per day. Energy requirements increase with mass.
- The model does not take into account competition between chicks or between adults and chicks and assumes no overlap in foraging.
- This model does not take into account any improved efficiency of feeding that may occur with age.
- The model assumes all accessible land is of equal value to the chicks.

6.2 Results of proposed risk assessment

The calculations refer only to the wader chicks as it is assumed the adult birds would be able to forage over such a large area that indirect effects of dip disposal by invertebrate prey depletion would be negligible.

Table 6.1: A summary of chick abilities, requirements and treated land productivity in terms of invertebrate food resources

Treatment Type	Age (days)	Mass (g)	ME (KJ)	Available Biomass (g/m^2)	Productivity (KJ/m^2)	Required Area (m^2)	Distance (m)	Possible Area (m^2)	No. of Chicks
OP	1	20	66.9	0.34	3.8	17.6	9.0	39.5	2
	3	30	96.7	0.34	3.8	25.5	26.9	355.8	14
	5	40	125.7	0.34	3.8	33.1	44.8	988.2	30
SP	1	20	66.9	0.97	10.9	6.1	9.0	39.5	6
	3	30	96.7	0.97	10.9	8.9	26.9	355.8	40
	5	40	125.7	0.97	10.9	11.5	44.8	988.2	86
OP dil	1	20	66.9	0.97	10.8	6.2	9.0	39.5	6
	3	30	96.7	0.97	10.8	8.9	26.9	355.8	40
	5	40	125.7	0.97	10.8	11.6	44.8	988.2	85
SP dil	1	20	66.9	0.80	8.9	7.5	9.0	39.5	5
	3	30	96.7	0.80	8.9	10.9	26.9	355.8	33
	5	40	125.7	0.80	8.9	14.1	44.8	988.2	70
Water	1	20	66.9	2.21	24.8	2.7	9.0	39.5	15
	3	30	96.7	2.21	24.8	3.9	26.9	355.8	91
	5	40	125.7	2.21	24.8	5.1	44.8	988.2	195

Age is the age of chick in days from hatching
Mass is the mass of the chick
ME is the energy the chick of a corresponding mass requires per day
Available Biomass is the average invertebrate biomass available per m^2 between disposal and 40 days
Productivity is the energy available in invertebrate matter in the treated areas
Required Area is the area a chick would need to occupy to get enough invertebrate food material
Distance is the distance a chick can move from the nest at a corresponding age and mass
Possible Area is the area a chick can forage over at the given age and mass, e.g. 0.157 of potential area
No. of Chicks is the number of chicks that can be supported for the given age and treatment type

Table 6.1 is a summary of the results at three different stages for each treatment type. This includes the mass of chick, energy and equivalent invertebrate biomass requirements, distance the chick can travel, productivity of the land with different treatment types and the area the chick would need to cover to obtain the energy requirements from each treatment type. The results show the lowest productivity is in the OP treated area at 3.8KJ/m^2 and the control, treated only with water has the highest productivity at 24.8KJ/m^2. The information given is for a single chick so does not take into account competition from other chicks in a brood or the requirements of the adult birds.

Figure 6.2 shows the number of chicks each treated area can sustain as the mobility, mass and energy requirements of the chicks increases with time. Appendix 7 shows the complete results of the numbers of chicks that can be supported at different mass/age on different treated areas. The control area, treated only with water, is the only treatment that could sustain 3 chicks for the first day, when a chick can cover an area of approximately 0.001ha, meaning it could probably support an average brood. By three days, the SP, SPdil, and OPdil treated areas could all support more than a single brood of chicks given the rapidly increasing mobility of the chicks and distance they can move from the nest. The OP treated site could not sustain a full brood until day four or five and this could therefore be expected to have the most deleterious impact on chick fitness and brood success.

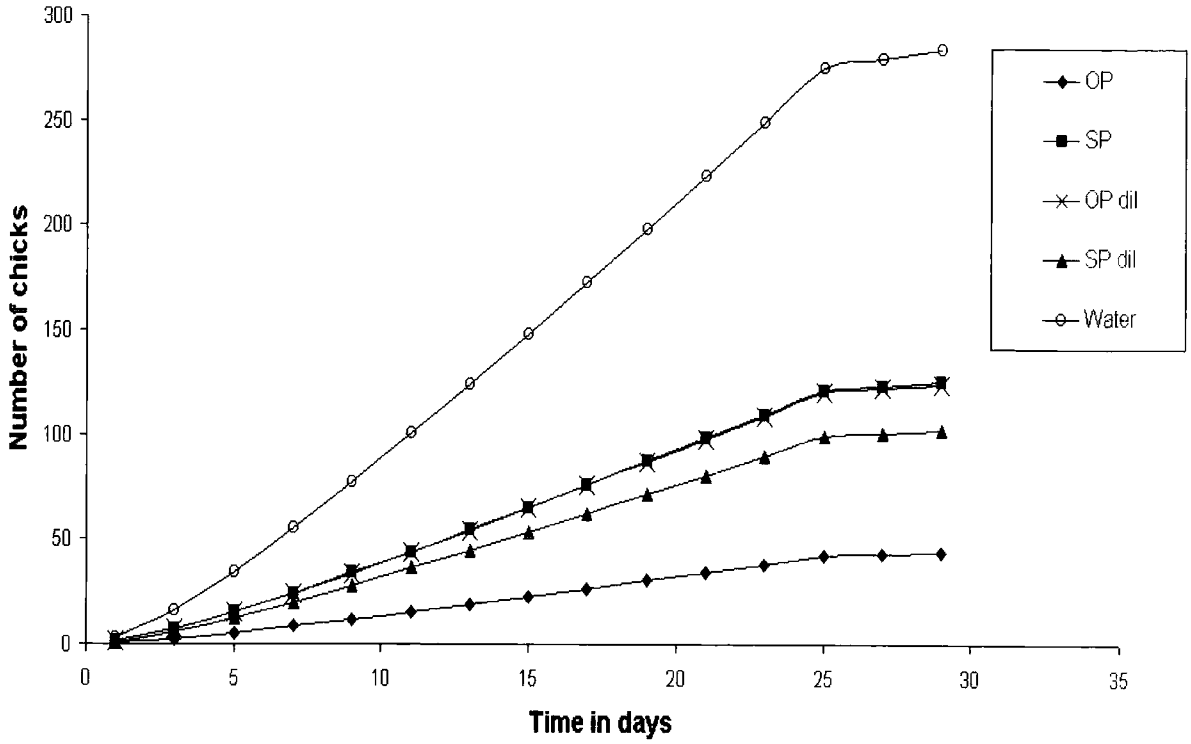


Figure 6.2: Number of chicks that can be sustained on sites subjected to spring applications of different dip treatments as chicks develop over time, with increasing size, energy requirements and home-range.

6.3 Application of risk assessment to a North Pennine Moors SPA

Background information for the North Pennine Moors case study, detailed below, was provided by the EA in September 2002. A map of the area, with the SPA and buffer zone marked, is included in Appendix 8. The buffer zone is added to account for birds nesting in the SPA that may travel considerable distances to feed.

<u>North Pennine Moors SPA Case Study</u>

- Eight authorisations for dip disposal within SPA
- 62 authorisations for dip disposal within 1km SPA
- Area of SPA = 30810 hectares
- Area of 1km buffer = 22980 ha
- Area of buffer + SPA = 53790 ha
- Average dip area = 2.7 ha
- Area of farmland that could receive dip within SPA is 21.6 ha
- Area of farmland that could receive dip within 1km buffer is 167.4 ha
- Area of farmland that could receive dip within SPA plus buffer is 189 ha
- Percentage of total area that could be used for dip disposal is 0.351 %

The above calculation assumes a worst case scenario that the entire area authorised for disposal will be utilised. Using the risk assessment equation: $\frac{KXFA}{ME} = N$ it can be established that a chick would have to be approximately 10 days old (approx.65g) to leave the disposal area if the nest is situated in the middle of the 2.7ha area, which means depending on the type and dilution of the dip applied it may not be able to move far enough at a younger age to obtain an adequate amount of invertebrates to maintain growth and development.

Assuming there are 35.3 breeding pairs of Lapwing per 100 ha, as found by Baines (1988) in a study of northern England, eight pairs could be at risk of breeding failure due to depletion of invertebrate populations within the 21.6 ha of dip disposal area in the North Pennine Moors SPA. This represents the annual potential loss of up to 23 chicks if the average clutch size is three eggs. On the entire SPA of 30810 ha there could be up to 10,876 breeding pairs, which could produce approximately 32,600 chicks per year. However, surveys by the RSPB (1999, 2002, 2003) suggest a decline in Lapwing densities of up to 50% between 1987 and 1998. Therefore, only approximately 16,300 chicks may currently be raised each year on the North Pennine Moors SPA. In addition, Baines (1988) suggested that 56% of Lapwing clutches were destroyed by farm machinery and predation, which further reduces the potential number of chicks raised on the SPA to less than 7,200 per year. Using these data the proportion of chicks that could be at risk from lowered invertebrate abundance due to dip disposal on the SPA is 0.07%, which means only five chicks are at risk. This is insignificant in terms of the whole breeding population.

Farmers may avoid applying for dip disposal permits on the SPA if they have alternative land in an unprotected area. Taking the 1km buffer zone into account the proportion of land used for dip disposal is greater. Incorporating the 50% decline sine 1987 (RSPB, 1999, 2002, 2003) and the 56% reduction due to farm machinery and predation (Baines, 1988) there could be up to 12,600 chicks raised on the SPA plus 1 km buffer from 4200 breeding pairs of lapwing. 200 of these chicks (1%) could be affected by invertebrate depletions on the 189 ha of dip disposal land, which is still insignificant in terms of the whole population. However, the calculations are based only on lapwing densities and the risk may be increased for species with smaller populations, in which individual birds are more important, and in areas where the proportion of land used for dip disposal is greater.

The above details a worst case scenario with the entire dip disposal area being used each time. This may not be the case as a typical residual volume of dip for disposal is approximately 1200 litres. With fourfold dilution and disposal via a vacuum tanker this may only cover 0.25 ha, which would result, for example, in only 17.5 ha out of the 53790 ha that makes up the SPA plus buffer zone for the case study being affected. This is less than 10% of the possible disposal area and can be taken as a best case scenario. The nest may also not be central in the disposal area, leading to faster possible escape to areas that may support a greater invertebrate population.

The position of the disposal sites in relation to each other is also important. The map (Appendix 8) shows that sites are not adjacent to each other on the SPA. This increases the chance of birds being able to leave a disposal area, if it has a depleted invertebrate population, and fulfil their dietary requirements elsewhere.

6.4 Discussion

Risk Assessment

The proposed risk assessment model was designed to be applicable over any area. However, the data used in the example was for rough grazing land at Sourhope Experimental farm and the biomass starting point may not be the same for other land use types. For example, Coulson and Whittaker (1978) found a low invertebrate biomass at

Moor House National Nature Reserve in the northern Pennines in comparison with lowland areas. Since invertebrate populations have been found to be depleted by dip disposal (Chapters 3 and 5) but not eliminated entirely it can be assumed that the greater the predisposal biomass, the greater the proportion of invertebrate biomass likely to be available after dip disposal. It is therefore important to assess the potential invertebrate biomass depending on area in the UK and land-use type and input data that is as accurate as possible for each scenario being investigated. Due to the limitations of the data used the results of this initial risk assessment are intended only as a rough guide to highlight factors involved in risk of dip disposal to upland wading birds.

The proposed risk assessment model has shown that, theoretically, young chicks, in an average brood of three, between one and three days from hatching might have difficulty in feeding over a large enough area to fulfil their daily metabolic energy requirements if their nests are within an area treated with OP based dips even if the dips are diluted to recommended levels (3 parts water to one part made-up dip). Although the disposal of undiluted sheep dip is a situation that should be avoided if EA recommendations are adhered to, it was apparent from answers to the questionnaires in Chapter 2 that dilution rates are not always accurately measured and can be greater or less than recommended levels depending on the disposal equipment a farmer has access to and the amount of dip to dispose of.

Although the results show that an average brood of three chicks might be able to collect enough food on any treatment site after the first few days from hatching, this does not take into account added pressure from the adult birds, any overlapping feeding areas from other nests or competition for surface invertebrates with other bird species and small mammals. The older the chick the greater its mass and also the greater its energy requirements, meaning it must move further in search of food. Therefore, with increasing age comes a greater chance of overlap with other feeding broods and increasing competition. The above model may therefore underestimate the possible negative effects on wading bird breeding success after the first few days of age. In natural populations nests are not spaced out evenly and some overlap in feeding areas would be expected. In northern Scotland, lapwings commonly nest in loose neighbourhood groups of 4 to 10 pairs, in nests between 10 and 150m apart, with wider spacing where food supply is poor (Cramp *et al.*, 1983).

The proposed risk assessment model shows that the depletion of available invertebrate biomass in disposal areas will necessitate chicks moving further and perhaps feeding for a longer time period in order to fulfil their dietary requirements. Baines (1988) found that food availability had only a minor effect on breeding success when compared to predation, soil moisture content and clutch destruction by farm machinery but adds that invertebrate biomass had probably surpassed a critical threshold level for the chicks during the study. Park *et al.* (2001) found that broods of red grouse *Lagopus lagopus scoticus* in which all the chicks survived between days four and ten had smaller home-range areas than broods in which some chicks died during the same period. The reason for increasing the home range was attributed to variations in invertebrate abundance that necessitated extending the foraging area.

Any additional difficulty in finding enough food because of depleted invertebrate densities may also exacerbate the problematic effects of other factors such as climate through the breeding season (Beintema and Visser, 1989a, 1989b). Below a certain temperature, dependant on body size, a chick must return to the nest to be brooded by parents at intervals to maintain body temperature. This therefore presents a further restriction in the distance a chick could move from the nest and since foraging is done in dry hours feeding time can be severely limited. In the worst case in prolonged adverse weather conditions many Lapwing chicks die of starvation (Beintema and Visser, 1989a). Therefore any reduction in invertebrate density could affect chick growth rate and even mortality, particularly when coupled with other adverse conditions.

Whittingham *et al.* (2000) attributed the selection, by foraging golden plovers, of only 17 out of 85 fields in the study area to high earthworm denstites, indicated by the presence of molehills in the preferred fields. Although earthworms have not been found to be affected by sheep dip disposal during this study, signs of depletion of other invertebrate taxa may influence habitat selection by other bird species, which could be of importance in designated protection areas where there are known traditional breeding areas.

The proposed risk assessment does not take into account any possible direct effects of dip disposal on upland birds that have been found in studies of similar pesticides (Hall, 1987). To avoid any possibility of direct poisoning by contaminated prey, spring disposal should not be made on areas known to be used by feeding waders, either chicks or adult birds) prior or during the breeding season.

Mitigating Risk

There are possibly several ways to minimise the risk of sheep dip disposal to the environment. The first is to add a chemical to the used dip at the end of the dipping process to speed up degradation of the active ingredients. In the case of one OP based sheep dip, for example, which contains the active ingredient Propetamphos, the manufacturer recommends adding sodium hypochlorite solution (10%) to the sheep dip wash, which it is claimed "rapidly degrades the OP insecticide within 24 hours." (Young's, 1999). For non OP dips containing High-cis Cypermethrin (SP) Young's recommend adding 5kg of sodium hydroxide and 5 litres of surfactant per 1000 litres of spent dip, which should degrade the insecticide within 12 hours. The claims of this and other dip manufacturers have not been tested as part of this investigation. However, if the claims of the manufacturer are realised in real life situations on farms this could be very important. Unfortunately, such choices currently rely on the purchaser of the sheep dip and their willingness to go to the additional expense of the degradation system in a process where the costs per head of sheep are already considerable.

The second method of reducing the risk of sheep dip disposal is to reduce the amount of dip that needs to be disposed. Mobile sheep dipping is an increasingly used alternative to the full immersion dipping process. Mobile dips create less waste dip and allow many sheep to be dealt with at the same time, leading to much faster throughput. For example, the "Monsoon" Mobile Sheep Shower (T.W. & L.A.Wilson) is a typical mobile system and allows a throughput of up to 225 sheep per hour. The sheep are first herded from an open pen into the main trailer-like enclosed holding pen a few at a time. Dip is sprayed at them from all angles for a set amount of time, after which they are released into an open holding pen that allows dip from the sheep to drain back into the system. The sheep can then be returned to the fields. The dip water becomes less soiled than in a traditional bath since any solid muck falling off the sheep is contained in the trailer and can be removed, rather than contaminating the dip water. Therefore fewer preservatives need to be added to stop bacterial growth etc. When a set number of sheep have been 'showered' the holding tank is topped up with more dip concentrate. When the amount of made-up dip in the holding tank reaches a minimum level it is topped up with both dip and fresh water from a clean water holding tank if more sheep await treatment.

At the end of a dipping session as little as 20 litres of spent dip may remain. This can either be disposed of onto farmland, or in the case of contractors or co-operatives can be incorporated into the next batch of freshly made-up dip, with waste disposed of only at the end of the dipping season. Co-operatives, where several farmers share the costs of a new mobile system, cut down on disposal volume, as disposal occurs once per co-operative dipping session rather than per farmer. For example, the National Trust have set up a trial scheme in Cumbria incorporating eight farms with land unsuitable for dip disposal due to possible contamination of adjoining bodies of water (National Trust pers. comm.). If the mobile dipping system proves as effective as the traditional dip bath method at protecting flocks long term, this could be an excellent method of allowing effective animal husbandry whilst keeping sheep dip disposal at an absolute minimum.

Whatever the amount of dip that remains for disposal, dip disposal must be carried out using practices that minimise risk to the environment. The findings of this investigation into the effects of sheep dip disposal on farmland have led to the following best practice recommendations that should minimise the risk of the future disposal of sheep dip to upland wading birds.

- Dip at any dilution should not be disposed on breeding sites during, or for at least 40 days prior to, the breeding season of the relevant wading birds (approx. April to June). During this time there are possible risks of both direct and indirect effects.

- Rough pasture, commonly used for dip disposal, is favoured nesting and feeding habitat for many wader species and so should be avoided for spring dip disposal, particularly if it is a known nesting site.

- Dip for disposal should always be diluted to the recommended levels.

- If possible within the authorised disposal area, the same area should not be used for disposal in consecutive years to allow the maximum possible time for recovery of invertebrate populations and minimise the possibility of persistence due to cumulative effects.

7. GENERAL DISCUSSION

This study has shown significant reductions in the densities of both active and sedentary soil invertebrates on areas where sheep dip disposal has taken place both in the Latin Square experiments (Chapter 5) and the farm site investigations (Chapter 3). The greatest reductions in invertebrate populations were found on areas that have been exposed to multiple disposals over a number of years such as at Derwent Reservoir and Yorkshire 4.

Short term effects that occur during any initial depletion of insect populations following pesticide application (Jepson, 1989) are mainly due to direct toxicity of the pesticide. Recovery is usually apparent by the following season after application of both organophosphates (Vickerman and Sunderland, 1977) and pyrethroids (Cole and Wilkinson, 1985; Shires, 1985). However, most studies do not determine whether recovery is due to recruitment from reproduction or recolonisation from adjacent untreated areas. If recovery is due to recolonisation from adjacent untreated areas widespread use of the pesticide may have a more significant effect than the studies suggest (Burn, 1989).

Although decreases in invertebrate density occurred as an immediate response to dip disposal in the Latin Square Experiments and on the farm sites, in some cases numbers of soil invertebrates remained lower on the disposal site than on the control area several seasons after dip application. Where farm sites had received multiple disposals or been in use for many years (Derwent and Yorkshire 4), the results were clear-cut, with marked reductions in densities of both sedentary and active soil organisms present in the spring, six months after the last disposal. This suggests persistence of toxic effects or slow rates of re-colonisation. Long-term invertebrate depletions can arise as a result of repeated applications of short-persistence chemicals, which impede any recovery that may occur after a single application, or as a result of a single application where recolonisation from adjacent land is poor (Burn, 1989).

Jepson (1989) speculated that delayed effects of pesticide application might occur in predatory species due to sublethal effects of the dip causing a reduction in activity, feeding and consequently fecundity. Long term reductions in density might also be experienced by predatory arthropods if their food supply had been diminished by previous insecticide applications (Jepson, 1989). Lowering of springtail densities after insecticide application could adversely affect densities of the many ground beetles that prey on them (Frampton, 1988), in particular the members of the *Leistus* and *Notiophilus* genera, which are Collembola specialists (Hengeveld, 1980). Depletion of other prey, such as Homoptera, that showed significant reductions in the Latin Square experiment at Sourhope, also reduce prey availability for predators such as Carabidae and Araneae. These indirect effects may be most apparent in subsequent seasons and are important but difficult to assess as a longer term risk.

In the present study there were more instances of depletion of sedentary invertebrates due to dip disposal than active invertebrates in both the farm sites and Latin Square experiments. Sedentary invertebrates are likely to be exposed to pesticides for longer periods and recolonisation rates are likely to be slower than for more active predatory invertebrates (Jepson, 1989). Depletion of sedentary invertebrates is likely to influence rates of recolonisation by active predators. However, since active predatory species are opportunistic feeders and can use alternative prey if some sources are depleted, absolute shortage of prey items is unlikely (Burn, 1989).

Analysis of the species composition of samples taken from the Latin Square Experiment at Sourhope, 2000-2001, and of pitfall catches on the farm sites, indicated that the susceptibility of the invertebrates exposed to dip varied, both between major taxa and at the species level. In particular, elaterid beetles and lycosid spiders showed a greater response to the presence of sheep dip than carabid beetles and linyphiid spiders. However, in other studies linyphiid spiders have been found to be particularly susceptible to synthetic pyrethroids pesticides (Frampton, 2001; Wiles and Jepson, 1992; Pullen *et al.*, 1992). Jepson (1989) suggested that 'the relative susceptibility of different species and life-stages to pesticides' is related to the degree of exposure to a pesticide and its residues, which is determined by the level of diel activity and the extent to which a species is plant or ground active. Brown *et al.* (1988) found that plant active Linyphiid spiders were affected by autumn pyrethroid application but ground active species were not. Moreby *et al.* (1997) also found that grass feeding species of non-target Heteroptera (including *Lepopterna dolabrata* and *Stenodema* spp.) were significantly depleted by pesticide applications whereas predatory species (including *Nabis* and *Anthocoris* spp.) displayed no response. These differing responses to pesticides between species are also reflected in vertebrates. Studies investigating shell thinning in birds due to pesticide contamination have found that some birds, particularly raptors and fish eating birds, are more susceptible than others due to a combination of ecological and physiological factors (Hall, 1987).

Although the broad conclusions above are probably justified, problems were encountered in attempting to quantify the effects of dip disposal. Similar problems have been encountered in some other studies on the effects of insecticides on non-target organisms. At a farm scale, other studies have found that the detection and duration of the effects of insecticide application depended on the size of plot studied (Jepson 1989; Pullen *et al.*, 1992). Plots smaller than 2 ha (larger than some of the disposal areas used in this study) were rapidly re-colonised by ground beetles and no effects of insecticide application could be detected in pitfall catches (Jepson, 1989). On 2 ha plots decreases in densities, after Dimethoate application, were detected up to seven days only (Fischer and Chambon, 1987). In the present study, sampling was delayed for at least 10 days after application in order to decrease risk to the investigator and, although this would depend on persistence, initial concentration of dip and susceptibility of different organisms, the evidence from Jepson's (1989) study suggests that this would allow time for reinvasion of the areas by mobile organisms. Reinvasion of treated plots from untreated plots has resulted in redistribution and an overall lowering of invertebrate density in previous studies of carabid beetles (Thacker, 1988) and linyphiid spiders (Thomas 1988). The reduced level and duration of statistically significant effects of pesticides can lead to underestimation of the impact of pesticide application (Jepson, 1989).

The apparent significance of initial depletions and subsequent detrimental effects of dip disposal on active invertebrates may be underestimated in this study, particularly if re-colonisation began prior to the first post disposal sampling, on sites in the farm investigation or the Latin Square experiment. When pitfall catches were made on the farms in spring in the present study, one site only had received dip in the same year (and showed a significant decrease in density of invertebrates after dip disposal), the others had had no dip disposed since the previous autumn. The detection of adverse effects at the community level in pitfall catches of ground beetles, but not spiders, may reflect the capacity for more rapid re-colonisation by the spiders (Wise, 1993). Re-colonisation by the soil fauna as a whole may account for the apparent lack of significant effects in soil samples at some of the sites in spring 2000.

The bird observations in this study provided confirmation that birds were feeding and nesting in fields where they could potentially be exposed to increased toxicity in prey and a reduction in prey availability as a result of dip disposal. The indirect effects of diminished invertebrate prey availability provided by some of the fields could affect breeding success in upland birds, as was found in a study on the Blue-tit *Parus caeruleus*, exposed to forest spraying with Cypermethrin (Pascual and Peris, 1992). Application of the pesticide caused nearly 100% mortality of lepidopteran larvae, an important food resource for breeding blue-tits. The nestling mortality of 81% on the treated plot compared with 6% on the control was attributed to a shortage in prey availability. Pesticides have also been found to alter bird feeding behaviour. Sublethal exposure of Red-winged Blackbirds *Agelaius phoeniceus* to the organophosphate pesticide parathion produced a longterm change in feeding behaviour and continued avoidance of untainted prey disrupted foraging and put breeding success at risk (Nicolaus and Lee, 1999). The Blackbirds in the treated territories consumed prey tainted with parathion up to three times before completely avoiding these prey due to conditioned taste aversion, even when parathion was no longer present.

The possible toxic effects of dip disposal on upland birds were not measured in this study but similar pesticides are associated with bird illness and mortality. Stone and Gradoni (1985) reported the death of over 700 geese due to one instance of spraying of organophosphate on the turf of a golf course in New York. Relatively recently Cobb *et al.* (2000) working on avian gastrointestinal tracts found that the dissipation of Diazinon from vegetation in the United States poses risks to passerines via ingestion of contaminated prey, although lethal exposures were limited to the day of application.

From the point of view of upland wader populations, the distinction between long and short-term effects is important. The Latin Square Experiments demonstrated the lethal effects of both Diazinon and Cypermethrin to the arthropods exposed on the vegetation and at the soil surface. In the first few days after hatching, the chicks of waders are restricted in their movements and dependent on surface-active arthropods (Baines 1990, Whittingham *et al.*, 2001). There was no recovery in density of Hemiptera in the treated plots at Sourhope, which are known to be an important food source for farmland birds (Moreby *et al.*, 1997). This is probably a combination of the relatively sedentary nature of many Hemiptera, leading to low levels of recolonisation and restriction in recruitment due to the single annual generation of adult Hemiptera that occurs in upland northern Britain (Whittaker, 1965). This demonstrates the importance of the timing of dip disposal, which could avoid this depletion of important food items. Dip should not be disposed during the period when young chicks are present when there are possible risks of both direct and indirect effects. The Farm Questionnaire (Chapter 2) revealed that by far the greatest proportion of the disposal areas were on rough pasture and, as this is favoured nesting habitat for many wader species (Stillman and Brown, 1998) it is particularly important to avoid spring disposal on these fields.

The possibility of sheep dips as soil pollutants has not been explored experimentally during this study but other studies suggest this may be an important aspect in assessing the overall damage to the environment of dip disposal, particularly when using repeated applications. Vink and van Straalen (1999) observed that Diazinon at 400 mu g/g reduced respiration, dehydrogenase and nitrification in isopod-mediated leaf litter decomposition in microcosms and sublethal effects of Diazinon on isopod (*Porcellionides pruinosus*) body growth have also been observed (Vink *et al.*, 1995). The synthetic pyrethroid sheep dip Bayticol has been found to cause up to four orders of magnitude increase in numbers of faecal coliforms and pathogens including *Salmonella* spp. (Semple *et al.*, 2000). This is particularly important since sheep dip can be diluted with slurry before disposal and is likely to come into contact with animal faeces once applied (Health and Safety Executive, 1998). The longevity of such pathogens may require extended periods of exclusion of farm animals from disposal areas and there is potential for increased numbers of pathogens to be transferred to animal and human foodchains and washed into aquatic ecosystems (Semple *et al.*, 2000).

The above pollution issues highlighted by Semple *et al.* (2000) may be addressed by recent research in which synthetic pyrethroid degrading bacteria have been isolated (*Pseudomonas* sp. and *Serratia* sp) that have the potential to be used in bioremediation of synthetic pyrethroid residues (Grant *et al.*, 2001). Experimental trials under laboratory conditions using the synthetic pyrethroid degrading organisms showed significant increases in synthetic pyrethroid breakdown when incubated at 25 ^{o}C with agitation at 80 rev min $^{-1}$ for 14 days (Grant and Betts, 2003). The authors believe the synthetic pyrethroid degrading bacteria could aid *in situ* or *ex situ* treatment of agricultural pesticide waste that would be preferable to current methods of incineration or disposal to land.

The absence of detectable effects on earthworm population densities in the present work is supported by other studies (O'Halloran *et al.*, 1999, for organophosphate based pesticide; Edwards and Brown, 1982, for synthetic pyrethroid studies). However, sublethal effects of exposure to organophosphate pesticide were shown experimentally in earthworms when cholinesterase activity was inhibited by 90% in worms exposed to dimethoate treated soil and had recovered only to 35% of the control level after 40 days (Dell'Omo *et al.*, 1999).

Despite evidence from laboratory studies showing reduced cholinesterase activity as a response to OP, no sublethal effects were detected in natural worm populations (Booth *et al.*, 2000). The earthworm population of the diet of adult lapwing in early spring is unlikely to be severely affected by the previous autumn's dip disposal.

Although depletion of natural earthworm populations as a result of sheep dip disposal seems unlikely, the possibility of secondary poisoning in vertebrates following exposure of the earthworms in their diet to pesticides is an important concern. Lumbricid earthworms can withstand higher levels of pesticide concentration in their tissues than many arthropods and can then deliver toxic levels of chemicals to susceptible predators (Rudd, 1964; Wallwork, 1976). This problem was illustrated when high levels of mortality were recorded in robins feeding on earthworms that lived in soil sprayed with DDT in the USA (Rudd, 1964; Carson, 1962). Earthworms can accumulate DDT in their tissues to a level ten times greater than in the sprayed soil (Wallwork, 1976). There is evidence to suggest that more recently produced pesticides may be similarly transferred to vertebrate predators by earthworms. When common shrews *Sorex araneus* were fed worms that had been exposed to the OP pesticide dimethoate the previous day the whole blood cholinesterase levels of the shrews was depressed to 64% of pre-exposure levels (Dell'Omo *et al.*, 1999). However, cholinesterase inhibition was not significant in shrews fed worms that had been exposed to dimethoate five and ten days previously.

Both lapwing and oystercatcher also feed to a large extent on leatherjackets (Baines 1980, Zwarts and Blomert, 1996). *Tipula paludosa*, the dominant pasture species, is present in the early instars through autumn into winter and a laboratory trial suggested it is likely to be vulnerable to autumn dip disposal (Butterfield, pers. Comm, Appendix 9). The dominant upland peat species *T. subnodicornis* showed high mortality on exposure to both Diazinon and Cypermethrin. The effects persisted for some time, with Diazinon causing significant mortality 17 days after application and an indication of sublethal toxicity lasting even longer. The laboratory experiment was subject to a number of problems and far removed from a realistic field trial. Whether tipulid species below the soil surface receive a lethal dosage from disposal in the field situation needs to be determined. Unfortunately, tipulid numbers were too low on the Latin Square Experimental sites to provide meaningful results for this group. However, the OP Chlorpyriphos is used as a spray for leatherjacket control (Hill, 1987) and there is no reason why the sheep dip application should be less efficient at soil penetration. Moreover the persistence of Diazinon is similar to Chlorpyriphos, with 50% remaining in non-sterile organic soil at 2 weeks and 2.5 weeks respectively (Verschueren, 1996). It has been suggested that dip disposal should not be made onto rough pasture where waders are nesting in spring, both because the invertebrates may be toxic and because the reduction in prey availability could be detrimental to the wader chicks. There are also grounds for suggesting that autumn disposal should not be made on improved ground where, typically, the larval densities of *T. paludosa* are higher than on the rough pasture. As *T. paludosa* has an annual life cycle there is no possibility of populations recovering before early spring, when large flocks of lapwing feed on improved pasture before dispersing to breed (Baines, 1990).

Long-term disposal onto breeding areas, whether in autumn or spring is probably the most damaging option. The earthworms on the improved pasture provide an important component of the diet in early spring and their densities are not affected by disposal. However, arthropods assume much greater importance on the rough pasture from May onwards when chicks are being reared (Baines, 1988). Although the persistence of both

Diazinon and Cypermethrin is low and many active species can re-colonise disposal areas, the densities of sedentary species in direct contact with the dip will be reduced. As this has the potential to reduce predator populations (Jepson, 1989), it is likely that the arthropod fauna as a whole will be affected, with a consequent reduction in food availability for birds.

This work has provided evidence that the disposal of both organophosphate and synthetic pyrethroid sheep dips onto farmland causes significant depletions of invertebrate populations. The longevity and severity of such depletions varies with the disposal regime but dip disposal on farmland is unlikely to cause large scale damage to terrestrial invertebrate communities in the long term. Additionally, the localised reductions in invertebrate prey for upland wading birds caused by current dip disposal are unlikely to pose a problem for adult birds due to their large potential foraging areas. However reductions in invertebrate prey are more important for chicks, which have more restricted movement and chick survival rates might be lowered if the home range area has to be expanded to fulfil dietary requirements (Park *et al.*, 2001). Sheep dip disposal is therefore a potential threat to chick survival and dip disposal on known breeding areas, particularly during the nesting season, should be avoided to minimise risk. These precautions are especially important in designated protection areas such as the study area in Teesdale where wading birds return annually to breed.

Despite the apparent risk to individual broods of upland wading birds as a result of invertebrate prey depletion, the North Pennine Moors SPA case study (Chapter 6) indicated that the proportion of birds potentially affected is very small and regional and national populations of upland wading bird species are not at risk from current dip disposal practices. Direct toxicity of sheep dip to birds was not addressed experimentally during this work but the possibility of birds being adversely affected by eating contaminated invertebrate prey is also currently a risk for individual birds rather than the population as a whole due to the relatively small scale of the dip disposal operation. The greatest risks to upland breeding populations of lapwings in particular are predation and destruction by farm machinery (Baines, 1988). Upland birds would only be at risk from dip disposal if populations were very small and the proportion of land used for disposal was increased substantially.

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9. APPENDICES

Appendix 1

Chi-square analyses for Teesdale 1A pre and post disposal soil sample data

Total Invertebrates

	control	disposal
pre-disposal	737.48	1193.07
post disposal	844.04	926.92
percentage difference	14	-22
χ^2	7.2	33.4

Beetles

	control	disposal
pre-disposal	243.59	274.94
post disposal	239.02	229.55
percentage difference	-2	-17
χ^2	0.0	4.1

Active Invertebrates

	control	disposal
pre-disposal	500.07	843.10
post disposal	491.76	528.98
percentage difference	-2	-37
χ^2	0.1	71.9

Flies

	control	disposal
pre-disposal	231.84	758.86
post disposal	390.86	423.51
percentage difference	69	-44
χ^2	40.6	95.1

Sedentary Invertebrates

	control	disposal
pre-disposal	254.69	332.41
post disposal	405.55	450.61
percentage difference	59	35
χ^2	34.5	17.8

Tipulid larvae

	control	disposal
pre-disposal	40.82	83.59
post disposal	254.69	282.45
percentage difference	524	238
χ^2	154.8	108.0

Appendix 2

Species Scores from Pitfall Catches 2000

Bugs

	Family	Species	T1C	T1E	T1AC	T1AE	T2C	T2E	T3C	T3E	T4C	T4E	T5C	T5E	T6C	T6E	Y3C	Y3E	Y4C	Y4E	Y5C	Y5E
1	Cicadellidae	unidentified nymphs											2	7	6	2	2	1				
2		Anoscopus albifrons																1	4			
3		Deltocephalus pulicaris												1		2						
4		Macrosteles sexnotatus														1						
5		Megopthalamus scanicus												1								
6		Planaphrodes bifasciata									2	3										
7		Psammotettix confusus												1								
8	Delphacidae	unidentified nymphs										1	1	2	1							
9		Javesella pellucida						2							1							
10		Muellerianella fairmairei												1					1			
11	Miridae	unidentified nymphs												1							1	
12	Saldidae	Saldula saltatoria							1			1	1									
13		Saldula scotica												1	1							
		number of species	0	0	0	0	0	1	1	0	1	3	3	8	4	3	1	2	2	0	1	0
		number of individuals	0	0	0	0	0	2	1	0	2	5	4	15	9	5	2	2	5	0	1	0

Spiders

	Family	Species		T1C	T1E	T1AC	T1AE	T2C	T2E	T3C	T3E	T4C	T4E	T5C	T5E	T6C	T6E	Y3C	Y3E	Y4C	Y4E	Y5C	Y5E
1	Amourobiidae	Coelotes atropos	A								1												
2	Linyphiidae	Agyneta decora	B													1		1	2				
3		Agyneta subtilis	B										2										
4		Baryphyma trifrons	B									1			1								
5		Bathyphantes gracilis	B					1		3	1	1	6	1	2	1	1			1	1	4	1
6		Centromerita concinna	B										1	1				2					
7		Ceratinella brevipes	B										2	4					1				
8		Dicymbium nigrum	B		2				8		4	7	4	11	11	6	11			3			
9		Dicymbium tibiale	B										2										
10		Diplocephalus latifrons	B	1				1															
11		Diplocephalus permixtus	B						1			9	4						1	1			
12		Dismodicus bifrons	B											1								1	
13		Erigone atra	B	11	15	12	13	26	17	17	13	30	23	7	8	6	5	4	13	20	6	33	19
14		Erigone dentipalpis	B	11	33	40	58	43	49	32	71	19	35	38	44	3	19	9	4	31	12	55	92
15		Erigone promiscua	B			1		2				1		1	1	12	2	10	4	5	3		1
16		Erigonella haemalis	B										9										
17		Gongylidium vivum	B							1				1									1
18		Hypomma bituberculatum	B				1	1				7	5	5	7	5	5				1		
19		Lepthyphantes ericaeus	B						1						1		3		1				
20		Lepthyphantes obscurus	B											2									
21		Lepthyphantes pallidus	B				1																
22		Lepthyphantes tenuis	B		1	1			1	1	5			2	9	4	5	1				1	
23		Lepthyphantes zimmermanni	B								2						1						
24		Leptorhoptrum robustum	B									1	1										
25		Lophomma punctatum	B									2	3										
26		Meioneta gulosa	B										1										
27		Meioneta rurestris	B																			2	
28		Meioneta saxatilis	B																			1	
29		Micrargus herbigradus	B										2	2	3	1							
30		Microlinyphia pusilla	B																1				
31		Milleriana inerrans	B																	1			
32		Oedothorax fuscus	B	4	5	1	3	12	24	72	37	21	17	170	117	25	64	5	10	14	20	69	15
33		Oedothorax gibbosus	B						1			2	2	2	2	1	1						
34		O. gibbosus f. tuberosus	B						1			1	5		2							1	
35		Oedothorax retusus	B		3		3	3		3	7	18	29	49	64	6	13	8	15	4	1	5	4
36		Pelecopsis mengei	B																				1
37		Pelecopsis parallela	B							26													
38		Pocadicnemis pumila	B									2							1		1	1	2
39		Porrhomma campbelli	B		1																		
40		Porrhomma montanum	B																				1
41		Savigina frontata	B			1			4	2	1	6					2		1				
42		Silometopus elegans	B		1			3	5		3	7	1	63	16	42	27		4		1	9	7
43		Tapinocyba pallens	B													1							
44		Tiso vagans	B											2	1		2						2
45		Typhochrestus digitatus	B															2					
46		Walckenaeria acuminata	B										1			2			1				
47		Walckenaeria nudipalpis	B											1				1		1			
48		Walckenaeria vigilax	B						1		1		1	6	4								
49	Lycosidae	Alopecosa pulverulenta	C									3		8	1	1		1	1				
50		Pardosa agricola	C		2		1	2	4		7	9	16	13	12	1		21	7	1		1	1
51		Pardosa amentata	C											7	3								
52		Pardosa nigriceps	C									1			1								
53		Pardosa pullata	C		1			1			4	37	11	53	17	9	3	24	16	1	1	1	3
54		Pirata piraticus	C										2	4	2	2							
55		Trochosa terricola	C											3	5			1			1		
56	Tetragnathidae	Pachygnatha degeeri	D						2	2		12	22	90	26	14	2	1	5				1
57	Theridiidae	Robertus lividus	E										1		1	1							2
58	Thomisidae	Ozyptila trux	F											1									
59		Xysticus cristatus	F											2	3			7	2			2	1
		number of species		4	10	6	7	11	14	10	14	22	27	29	27	21	17	16	19	12	11	15	17
		number of individuals		27	64	56	80	95	119	159	157	197	208	550	364	144	166	98	90	83	48	186	154

Foraging Guild Key:

A = Funnel Web
B = Sheet Line Weavers
C = Diurnal Running Spiders
D = Orb Weavers
E = Scattered Line Weavers
F = Crab Spiders

Carabids

	Species	T1C	T1E	T1AC	T1AE	T2C	T2E	T3C	T3E	T4C	T4E	T5C	T5E	T6C	T6E	Y3C	Y3E	Y4C	Y4E	Y5C	Y5E
1	*Carabus problematicus*															3	4	1			
2	*C. violaceus*															2	3				
3	*Leistus rufescens*							1													
4	*Nebria brevicollis*	13	35	23	20	74	57	41	295	7	18	1	3		13	2	6	24	25		2
5	*N. salina*	1						2		1		1	1		2	66	31	3	3		1
6	*Notiophilus aquaticus*											2			1	4	3				
7	*N. biguttatus*										2										
8	*N. substriatus*											1				1	3				
9	*Elaphrus riparius*									1										1	
10	*Loricera pilicornis*	5	28	12	13	8	29	4	21	25	31	36	11	24	12	3	15	3	5	18	3
11	*Dyschirius globosus*												1	3	1	4	10			7	13
12	*Clivina fossor*	2	4	2		1	5		3				1					2	1	3	16
13	*Miscodera arctica*															2					
14	*Patrobus assimilis*											5	11	1	4						
15	*P. atrorufus*	5	1	1	1		7	3	3	2		2	4								
16	*Trechus obtusus*												1								
17	*Bembidion aeneum*									1	2										
18	*B. guttula*		1	1							3										
19	*B. lampros*															11	4	1	14	2	4
20	*B. lunulatum*																			1	2
21	*B. nigricorne*												1								
22	*B. unicolor*									2	3		3								
23	*Pterostichus adstrictus*															17	6				
24	*P. diligens*									1		12	2	10	15	3	1	5			
25	*P. madidus*								1				2		1	1		121	3		32
26	*P. melanarius*	1		18	1	23	16		14		1										
27	*P. nigrita agg.*									3	2	88	8	155	22		3	1		4	1
28	*P. strenuus*					1	1		3	2	6	11	14	3	5	3	9			2	3
29	*Calathus fuscipes*															7	1	3			3
30	*C. melanocephalus*															2		15	2		8
31	*Synuchus nivalis*				1										1						
32	*Agonum fuliginosum*										1										
33	*A. muelleri*		4			1			1	3	2		2				1	1	4	10	
34	*A. aenea*																	1	39	1	21
35	*A. aulica*												1								
36	*A. familiaris*												1								
37	*A. lunicollis*																				9
38	*A. plebeja*																				10
39	*T. placidus*													1							
	number of species	6	6	6	5	6	6	5	8	11	11	10	17	7	11	16	15	13	9	10	16
	number of individuals	27	73	57	36	108	115	51	341	48	71	159	67	197	77	131	100	181	96	49	129

Appendix 3

Results of ANOVA and Tukey HSD tests from the Latin Square Experimental Site, Sourhope 2000-2001

Results of ANOVA based on the total number of invertebrates found in soil samples predisposal and at 10, 20 and 40 days and 12 months after sheep dip disposal at Sourhope, 2000

4.1.1

Pre-disposal	means/ m^2	Anova $F_{4, 20}$	Anova Significant	10 day	means/ m^2	Anova $F_{4, 20}$	Anova Significant
sp dil	601.6	0.10	N/S	sp dil	656.0	2.34	N/S
op	521.6			op	396.8		
sp	492.8			sp	563.2		
op dil	560.0			op dil	816.0		
water	582.4			water	867.2		

4.1.2

20 day	means/ m^2	Anova $F_{4, 20}$	Anova Significant	40 day	means/ m^2	Anova $F_{4, 20}$	Anova Significant
sp dil	630.4	1.50	N/S	sp dil	432.0	0.94	N/S
op	601.6			op	336.0		
sp	684.8			sp	364.8		
op dil	508.8			op dil	396.8		
water	825.6			water	540.8		

4.1.3

12 month	means/ m^2	Anova $F_{4, 16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	627.2	**3.68**	Yes	sp dil. vs op	**reject H_0**
op	1113.6		$P<0.05$	sp dil. vs sp	accept H_0
sp	483.2			sp dil. vs op dil.	**reject H_0**
op dil	1177.6			sp dil. vs water	accept H_0
water	1027.2			op vs sp	**reject H_0**
				op vs op dil.	accept H_0
				op vs water	accept H_0
				sp vs op dil.	**reject H_0**
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

Results of ANOVA based on the number of sedentary invertebrates found in soil samples predisposal and at 10, 20 and 40 days and 12 months after sheep dip disposal at Sourhope, 2000

4.1.4

Pre-disposal	means/ m^2	Anova $F_{4, 20}$	Anova Significant
sp dil	400.0	0.12	N/S
op	284.8		
sp	252.8		
op dil	336.0		
water	284.8		

4.1.5

10 day	means/ m^2	Anova $F_{4, 16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	323.2	**3.39**	Yes	sp dil. vs op	accept H_0
op	160.0		$P<0.05$	sp dil. vs sp	accept H_0
sp	252.8			sp dil. vs op dil.	accept H_0
op dil	236.8			sp dil. vs water	accept H_0
water	483.2			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	accept H_0
				op dil. vs water	accept H_0

4.1.6

20 day	means/ m^2	Anova $F_{4, 20}$	Anova Significant	40 day	means/ m^2	Anova F	Anova Significant
sp dil	342.4	1.52	N/S	sp dil	156.8	1.09	N/S
op	262.4			op	9.2		
sp	313.6			sp	9.6		
op dil	236.8			op dil	10		
water	518.4			water	17.2		

4.1.7

12 month	means/ m^2	Anova $F_{4, 20}$	Anova Significant
sp dil	451.2	2.31	N/S
op	806.4		
sp	326.4		
op dil	822.4		
water	697.6		

Results of ANOVA based on the total number of invertebrates caught in pitfall traps at 10, 20 and 40 days and 12 months after sheep dip disposal at Sourhope, 2000

4.2.1

10 day	means/ sample	Anova $F_{4, 20}$	Anova Significant
sp dil	33.6	1.39	N/S
op	25.2		
sp	28.8		
op dil	36.6		
water	48.0		

4.2.2

20 day	means/ sample	Anova $F_{4, 16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	19.6	**4.24**	Yes	sp dil. vs op	accept H_0
op	13.4		P<0.05	sp dil. vs sp	accept H_0
sp	10.4			sp dil. vs op dil.	accept H_0
op dil	15.6			sp dil. vs water	accept H_0
water	23.0			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	accept H_0
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

4.2.3

40 day	means/ sample	Anova $F_{4, 20}$	Anova Significant
sp dil	39.6	1.10	N/S
op	33.0		
sp	39.6		
op dil	34.6		
water	44.6		

4.2.4

12 months	means/ sample	Anova $F_{4, 20}$	Anova Significant
sp dil	84.6	0.38	N/S
op	89.0		
sp	85.6		
op dil	84.4		
water	94.0		

Results of ANOVA based on the number of Linyphiidae caught in pitfall traps at 10, 20 and 40 days after sheep dip disposal at Sourhope, 2000

4.2.5

10 day	means/ sample	Anova $F_{4, 20}$	Anova Significant
sp dil	4.0	2.10	N/S
op	5.0		
sp	1.4		
op dil	4.0		
water	6.0		

4.2.6

20 day	means/ sample	Anova $F_{4, 20}$	Anova Significant
sp dil	2.0	1.28	NS
op	2.0		
sp	0.2		
op dil	2.0		
water	1.6		

4.2.7

40 day	means/ sample	Anova $F_{4, 20}$	Anova Significant
sp dil	5.60	2.46	NS
op	2.60		
sp	2.20		
op dil	3.00		
water	3.80		

Results of ANOVA based on the number of Lycosidae caught in pitfall traps at 10, 20 and 40 days after sheep dip disposal at Sourhope, 2000

4.2.8

10 day	means/ sample	Anova $F_{4, 20}$	Anova Significant
sp dil	0.8	2.64	NS
op	1.4		
sp	3.0		
op dil	4.4		
water	7.0		

4.2.9

20 day	means/ sample	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	0.2	**25.37**	Yes	sp dil. vs op	**reject H_0**
op	1.0		P<0.01	sp dil. vs sp	accept H_0
sp	0.2			sp dil. vs op dil.	**reject H_0**
op dil	1.4			sp dil. vs water	**reject H_0**
water	2.2			op vs sp	**reject H_0**
				op vs op dil.	accept H_0
				op vs water	**reject H_0**
				sp vs op dil.	**reject H_0**
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

4.2.10

40 day	means/ sample	Anova $F_{4, 16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	0.6	**5.19**	Yes	sp dil. vs op	accept H_0
op	2.6		P<0.01	sp dil. vs sp	accept H_0
sp	0.0			sp dil. vs op dil.	accept H_0
op dil	2.0			sp dil. vs water	accept H_0
water	2.2			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	accept H_0
				sp vs op dil.	**reject H_0**
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

Results of ANOVA based on the number of elaterids caught in pitfall traps at 10, 20 and 40 days after sheep dip disposal at Sourhope, 2000

4.2.11

10 day	means/ sample	Anova $F_{4, 20}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	5.0	**2.94**	Yes	sp dil. vs op	**reject H_0**
op	0.6		P<0.05	sp dil. vs sp	accept H_0
sp	2.8			sp dil. vs op dil.	accept H_0
op dil	6.2			sp dil. vs water	accept H_0
water	8.0			op vs sp	**reject H_0**
				op vs op dil.	**reject H_0**
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

4.2.12

20 day	means/ sample	Anova $F_{4, 20}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	1.4	**3.09**	Yes	sp dil. vs op	accept H_0
op	0.6		P<0.05	sp dil. vs sp	accept H_0
sp	0.2			sp dil. vs op dil.	accept H_0
op dil	1.8			sp dil. vs water	accept H_0
water	3.6			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	accept H_0
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

4.2.13

40 day	means/ sample	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	2.2	**4.11**	Yes	sp dil. vs op	accept H_0
op	0.4		P<0.05	sp dil. vs sp	accept H_0
sp	0.6			sp dil. vs op dil.	accept H_0
op dil	2.4			sp dil. vs water	accept H_0
water	3.6			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	accept H_0
				op dil. vs water	accept H_0

Results of ANOVA based on the number of carabids caught in pitfall traps at 10, 20 and 40 days after sheep dip disposal at Sourhope, 2000

4.2.14

10 day	means/ sample	Anova $F_{4, 20}$	Anova Significant
sp dil	2.0	0.51	N/S
op	0.2		
sp	0.8		
op dil	1.2		
water	1.4		

4.2.15

20 day	means/ sample	Anova $F_{4, 20}$	Anova Significant
sp dil	2.0	1.53	N/S
op	0.6		
sp	1.6		
op dil	1.0		
water	0.6		

4.2.16

40 day	means/ sample	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	4.6	**4.02**	Yes	sp dil. vs op	accept H_0
op	2.6		$P<0.05$	sp dil. vs sp	accept H_0
sp	3.4			sp dil. vs op dil.	accept H_0
op dil	1.4			sp dil. vs water	**reject H_0**
water	0.2			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	accept H_0
				sp vs op dil.	accept H_0
				sp vs water	accept H_0
				op dil. vs water	accept H_0

sp contains more carabids than the water treatment where the Tukey test rejects H_0

Results of ANOVA based on the total number of invertebrates caught by suction sampling at 10 and 40 days after sheep dip disposal at Sourhope, 2000

4.3.1

10 day	means/ sample	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion	40 day	means	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	17.8	**17.48**	Yes	spd vs op	accept H_0	sp dil	16.4	**17.50**	Yes	spd vs op	accept H_0
op	14.2		P<0.05	spd vs sp	accept H_0	op	12.6		P<0.05	spd vs sp	accept H_0
sp	13.0			spd vs opd	accept H_0	sp	10.4			spd vs opd	accept H_0
op dil	11.8			spd vs water	**reject H_0**	op dil	12.4			spd vs water	**reject H_0**
water	81.2			op vs sp	accept H_0	water	44.6			op vs sp	accept H_0
				op vs opd	accept H_0					op vs opd	accept H_0
				op vs water	**reject H_0**					op vs water	**reject H_0**
				sp vs opd	accept H_0					sp vs opd	accept H_0
				sp vs water	**reject H_0**					sp vs water	**reject H_0**
				opd vs water	**reject H_0**					opd vs water	**reject H_0**

Results of ANOVA based on the total number of bugs caught by suction sampling at 10 and 40 days after sheep dip disposal at Sourhope, 2000

4.3.2

10 day	means/ sample	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion	40 day	means	Anova $F_{4, 12}$	Anova Significant	Comparison	conclusion
sp dil	5.0	**20.55**	Yes	sp dil. vs op	accept H_0	sp dil	9.8	**15.35**	Yes	sp dil. vs op	accept H_0
op	5.4		P<0.05	sp dil. vs sp	accept H_0	op	3.0		P<0.05	sp dil. vs sp	accept H_0
sp	3.6			sp dil. vs op dil.	accept H_0	sp	4.0			sp dil. vs op dil.	accept H_0
op dil	4.8			sp dil. vs water	**reject H_0**	op dil	4.0			sp dil. vs water	**reject H_0**
water	54.4			op vs sp	accept H_0	water	32.6			op vs sp	accept H_0
				op vs op dil.	accept H_0					op vs op dil.	accept H_0
				op vs water	**reject H_0**					op vs water	**reject H_0**
				sp vs op dil.	accept H_0					sp vs op dil.	accept H_0
				sp vs water	**reject H_0**					sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**					op dil. vs water	**reject H_0**

Results of ANOVA based on the number of *Hyledelphax elegantulus* caught by suction sampling at 10 days after sheep dip disposal at Sourhope, 2000

4.3.3

10 day	means/ sample	Anova $F_{4, 20}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	6.4	**2.96**	Yes	sp dil. vs op	accept H_0
op	1.0		P<0.05	sp dil. vs sp	accept H_0
sp	0.6			sp dil. vs op dil.	accept H_0
op dil	1.0			sp dil. vs water	**reject H_0**
water	12.2			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

Results of ANOVA based on the number of *Pachytomella parallela* caught by suction sampling at 40 days after sheep dip disposal at Sourhope, 2000

4.3.4

40 day	means/ sample	Anova $F_{4, 20}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	7.0	**32.95**	Yes	sp dil. vs op	accept H_0
op	1.4		P<0.01	sp dil. vs sp	accept H_0
sp	3.4			sp dil. vs op dil.	accept H_0
op dil	3.4			sp dil. vs water	**reject H_0**
water	28.2			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

Results of ANOVA and Tukey HSD tests from the Latin Square Experimental Site, Newton Rigg 2001-2002

Results of ANOVA based on the total number of invertebrates found in soil samples predisposal and at 10 days after sheep dip disposal at Newton Rigg, 2001

1.2

Pre-disposal	means/ m^2	Anova $F_{4,16}$	Anova Significant
sp dil	1505.40	0.54	N/S
op	1869.04		
sp	1301.82		
op dil	1507.78		
water	2001.76		

1.3

10 day	means/ m^2	Anova $F_{4,16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	1051.05	**8.65**	Yes	sp dil. vs op	**reject H_0**
op	333.65		$P<0.05$	sp dil. vs sp	**reject H_0**
sp	542.53			sp dil. vs op dil.	accept H_0
op dil	707.59			sp dil. vs water	accept H_0
water	1153.22			op vs sp	**reject H_0**
				op vs op dil.	**reject H_0**
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

Results of ANOVA based on the number of sedentary invertebrates found in soil samples predisposal and at 10 days after sheep dip disposal at Newton Rigg, 2001

1.4

Pre-disposal	means/ m^2	Anova $F_{4,16}$	Anova Significant
sp dil	468.99	0.37	N/S
op	637.33		
sp	414.85		
op dil	440.06		
water	769.30		

1.5

10 day	means/ m^2	Anova $F_{4, 16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	265.25	**7.88**	Yes	sp dil. vs op	**reject H_0**
op	86.48		P<0.05	sp dil. vs sp	accept H_0
sp	190.37			sp dil. vs op dil.	accept H_0
op dil	251.10			sp dil. vs water	accept H_0
water	437.95			op vs sp	**reject H_0**
				op vs op dil.	**reject H_0**
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

1.7

10 day	means/ m^2	Anova $F_{4, 16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	644.97	**4.41**	Yes	sp dil. vs op	**reject H_0**
op	126.47		P<0.05	sp dil. vs sp	**reject H_0**
sp	247.99			sp dil. vs op dil.	**reject H_0**
op dil	281.41			sp dil. vs water	accept H_0
water	487.89			op vs sp	**reject H_0**
				op vs op dil.	**reject H_0**
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

Results of ANOVA based on the number of active invertebrates found in soil samples predisposal and at 10 days after sheep dip disposal at Newton Rigg, 2001

1.6

Pre-disposal	means/ m^2	Anova $F_{4, 16}$	Anova Significant
sp dil	832.89	0.77	N/S
op	843.14		
sp	511.37		
op dil	609.42		
water	1110.75		

Results of ANOVA based on the total number of earthworms found in soil samples predisposal and at 10 days after sheep dip disposal at Newton Rigg, 2001

1.8 **1.9**

Pre-disposal	means/ m^2	Anova $F_{4, 12}$	Anova Significant	10 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	761.74	0.66	N/S	sp dil	600.54	0.81	N/S
op	1082.15			op	380.29		
sp	1131.08			sp	594.32		
op dil	1192.52			op dil	645.15		
water	894.36			water	730.02		

Results of ANOVA based on the total number of invertebrates caught in pitfall traps at 10 days after sheep dip disposal at Newton Rigg, 2001

1.10

10 day	means/ sample	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	15.16	**4.05**	Yes	sp dil. vs op	accept H_0
op	27.93		$P<0.05$	sp dil. vs sp	accept H_0
sp	13.89			sp dil. vs op dil.	accept H_0
op dil	21.73			sp dil. vs water	accept H_0
water	19.99			op vs sp	**reject H_0**
				op vs op dil.	accept H_0
				op vs water	accept H_0
				sp vs op dil.	**reject H_0**
				sp vs water	accept H_0
				op dil. vs water	accept H_0

Results of ANOVA based on the total number of invertebrates found in soil samples predisposal and at 10, 20 and 40 days after sheep dip disposal at Newton Rigg Site A, 2002

1.11 **1.12**

Pre-disposal	means/ m^2	Anova $F_{4, 12}$	Anova Significant	10 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	1426	0.22	N/S	sp dil	722.00	1.39	N/S
op	1295			op	550.00		
sp	1441			sp	634.00		
op dil	1384			op dil	750.00		
water	1298			water	1332.00		

1.13

20 day	means/ m^{-}	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	601	**4.02**	Yes	sp dil. vs op	accept H_0
op	610		P<0.05	sp dil. vs sp	**reject H_0**
sp	732			sp dil. vs op dil.	**reject H_0**
op dil	1015			sp dil. vs water	**reject H_0**
water	1275			op vs sp	accept H_0
				op vs op dil.	**reject H_0**
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

1.14

40 day	means/ m^{-}	Anova $F_{4, 12}$	Anova Significant
sp dil	687	2.06	N/S
op	699		
sp	491		
op dil	975		
water	1078		

Results of ANOVA based on the number of sedentary invertebrates found in soil samples predisposal and at 10, 20 and 40 days after sheep dip disposal at Newton Rigg Site A, 2002

1.15 **1.16**

Pre-disposal	means/ m^{2}	Anova $F_{4, 12}$	Anova Significant	10 day	means/ m^{2}	Anova $F_{4, 16}$	Anova Significant
sp dil	731	0.38	N/S	sp dil	152	2.88	N/S
op	634			op	200		
sp	806			sp	288		
op dil	680			op dil	303		
water	606			water	439		

1.17

20 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	207	**9.56**	Yes	sp dil. vs op	accept H_0
op	206		P<0.05	sp dil. vs sp	**reject H_0**
sp	92			sp dil. vs op dil.	accept H_0
op dil	308			sp dil. vs water	**reject H_0**
water	415			op vs sp	**reject H_0**
				op vs op dil.	**reject H_0**
				op vs water	**reject H_0**
				sp vs op dil.	**reject H_0**
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

1.18

40 day	means/ m^2	Anova $F_{4, 16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	500	**3.49**	Yes	sp dil. vs op	accept H_0
op	490		P<0.05	sp dil. vs sp	**reject H_0**
sp	284			sp dil. vs op dil.	accept H_0
op dil	538			sp dil. vs water	accept H_0
water	437			op vs sp	**reject H_0**
				op vs op dil.	accept H_0
				op vs water	accept H_0
				sp vs op dil.	**reject H_0**
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

Results of ANOVA based on the number of active invertebrates found in soil samples predisposal and at 10 , 20 and 40 days after sheep dip disposal at Newton Rigg Site A, 2002

1.19

Pre-disposal	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	767	0.12	N/S
op	637		
sp	715		
op dil	745		
water	713		

1.20

10 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	632	0.14	N/S
op	352		
sp	296		
op dil	488		
water	878		

1.21

20 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	416	2.36	N/S
op	441		
sp	626		
op dil	718		
water	890		

1.22

40 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	223	1.08	N/S
op	231		
sp	242		
op dil	558		
water	673		

Results of ANOVA based on the total number of invertebrates found in soil samples predisposal and at 10, 20 and 40 days after sheep dip disposal at Newton Rigg Site B, 2002

1.23

Pre-disposal	means/ m^2	Anova $F_{4,12}$	Anova Significant
sp dil	1867	0.76	N/S
op	1561		
sp	1501		
op dil	1287		
water	1768		

1.24

10 day	means/ m^2	Anova $F_{4,12}$	Anova Significant
sp dil	707	2.05	N/S
op	739		
sp	598		
op dil	661		
water	1309		

1.25

20 day	means/ m^2	Anova $F_{4,16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	833	**5.70**	Yes	sp dil. vs op	**reject H_0**
op	479		$P<0.05$	sp dil. vs sp	**reject H_0**
sp	427			sp dil. vs op dil.	accept H_0
op dil	913			sp dil. vs water	**reject H_0**
water	1361			op vs sp	accept H_0
				op vs op dil.	**reject H_0**
				op vs water	**reject H_0**
				sp vs op dil.	**reject H_0**
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

1.26

40 day	means/ m^2	Anova $F_{4,12}$	Anova Significant
sp dil	740	1.41	N/S
op	1202		
sp	612		
op dil	1001		
water	1177		

Results of ANOVA based on the number of sedentary invertebrates found in soil samples predisposal and at 10, 20 and 40 days after sheep dip disposal at Newton Rigg Site B, 2002

1.27

Pre-disposal	means/ m^2	Anova $F_{4,12}$	Anova Significant
sp dil	1047	0.30	N/S
op	898		
sp	1016		
op dil	912		
water	982		

1.28

10 day	means/ m^2	Anova $F_{4, 16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	283	**5.88**	Yes	sp dil. vs op	**reject H_0**
op	226		P<0.05	sp dil. vs sp	accept H_0
sp	237			sp dil. vs op dil.	**reject H_0**
op dil	135			sp dil. vs water	**reject H_0**
water	733			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	**reject H_0**
				sp vs op dil.	**reject H_0**
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

1.29

20 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	308	**8.49**	Yes	sp dil. vs op	**reject H_0**
op	221		P<0.05	sp dil. vs sp	**reject H_0**
sp	188			sp dil. vs op dil.	accept H_0
op dil	260			sp dil. vs water	**reject H_0**
water	632			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

1.30

40 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	441	1.75	N/S
op	589		
sp	331		
op dil	732		
water	612		

Results of ANOVA based on the number of active invertebrates found in soil samples predisposal and at 10 , 20 and 40 days after sheep dip disposal at Newton Rigg Site B, 2002

1.31 **1.32**

Pre-disposal	means/ m^2	Anova $F_{4, 12}$	Anova Significant	10 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	862	2.46	N/S	sp dil	448	1.31	N/S
op	679			op	395		
sp	521			sp	456		
op dil	433			op dil	566		
water	792			water	656		

1.34 **1.35**

20 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant	40 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	622	2.18	N/S	sp dil	329	1.14	N/S
op	299			op	635		
sp	262			sp	315		
op dil	686			op dil	283		
water	767			water	781		

Results of ANOVA based on the number of active invertebrates found in soil samp
10, 20 and 40 days after sheep dip disposal at Newton Rigg Site A+B, 2002

1.36

10 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	1018	1.79	N/S
op	637		
sp	606		
op dil	1002		
water	1465		

1.37

20 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	936	**4.48**	Yes	sp dil. vs op	accept H_0
op	729		$P<0.05$	sp dil. vs sp	accept H_0
sp	843			sp dil. vs op dil.	**reject H_0**
op dil	1360			sp dil. vs water	**reject H_0**
water	1574			op vs sp	**reject H_0**
				op vs op dil.	**reject H_0**
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	accept H_0

1.38

40 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	509	1.91	N/S
op	828		
sp	494		
op dil	655		
water	1288		

Site A: Results of ANOVA based on the total number of earthworms found in soil samples predisposal and at 10, 20 and 40 days after sheep dip disposal at Newton Rigg, 2002

1.39

Pre-disposal	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	414	1.25	N/S
op	426		
sp	381		
op dil	523		
water	310		

1.40

10 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	295	0.29	N/S
op	351		
sp	273		
op dil	299		
water	352		

1.41

20 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	285	0.93	N/S
op	191		
sp	199		
op dil	259		
water	214		

1.42

40 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	282	**3.56***	Yes	sp dil. vs op	**reject H_0**
op	176		$P<0.05$	sp dil. vs sp	accept H_0
sp	242			sp dil. vs op dil.	accept H_0
op dil	264			sp dil. vs water	**reject H_0**
water	183			op vs sp	accept H_0
				op vs op dil.	**reject H_0**
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

NB * denotes significantly higher numbers on the treated sites than the control (water)

Site B: Results of ANOVA based on the total number of earthworms found in soil samples predisposal and at 10, 20 and 40 days after sheep dip disposal at Newton Rigg, 2002

1.43

Pre-disposal	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	565	0.99	N/S
op	449		
sp	493		
op dil	434		
water	568		

1.44

10 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	429	0.24	N/S
op	377		
sp	300		
op dil	428		
water	377		

1.45

20 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	321	2.00	N/S
op	232		
sp	285		
op dil	301		
water	340		

1.46

40 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	304	0.38	N/S
op	244		
sp	271		
op dil	183		
water	176		

Results of ANOVA based on the total number of invertebrates found in suction samples 50 days after sheep dip disposal at Newton Rigg Site A, 2002

1.47

50 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	936	0.98	N/S
op	786		
sp	751		
op dil	648		
water	916		

Results of ANOVA based on the total number of invertebrates found in suction samples 50 days after sheep dip disposal at Newton Rigg Site B, 2002

1.48

50 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	1157	0.83	N/S
op	1151		
sp	988		
op dil	1340		
water	1292		

Results of ANOVA based on the number of collembola found in suction samples 50 days after sheep dip disposal at Newton Rigg Site A, 2002

1.49

50 day	means/ m^{-}	Anova $F_{4, 12}$	Anova Significant
sp dil	1406	1.56	N/S
op	1000		
sp	2077		
op dil	996		
water	1736		

Results of ANOVA based on the number of collembola found in suction samples 50 days after sheep dip disposal at Newton Rigg Site B, 2002

1.50

50 day	means/ m^{-}	Anova $F_{4, 12}$	Anova Significant
sp dil	585	0.25	N/S
op	244		
sp	406		
op dil	553		
water	512		

Results of ANOVA based on the number of mites found in suction samples 50 days after sheep dip disposal at Newton Rigg Site A, 2002

1.51

50 day	means/ m^{-}	Anova $F_{4, 20}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	778	**6.73**	Yes	sp dil. vs op	**reject H_0**
op	1450		$P<0.05$	sp dil. vs sp	accept H_0
sp	863			sp dil. vs op dil.	**reject H_0**
op dil	1381			sp dil. vs water	**reject H_0**
water	3548			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

Results of ANOVA based on the number of mites found in suction samples
50 days after sheep dip disposal at Newton Rigg Site B, 2002

1.52

50 day	means/ m^2	Anova $F_{4,12}$	Anova Significant
sp dil	899	2.28	N/S
op	1859		
sp	781		
op dil	2508		
water	1166		

Results of ANOVA based on the total number of invertebrates found in soil cores
predisposal and at 10, 20 and 40 days after sheep dip disposal at Newton Rigg Site B, 2002

2.1 **2.2**

Pre-disposal	means/ m^2	Anova $F_{4,12}$	Anova Significant	10 day	means/ m^2	Anova $F_{4,12}$	Anova Significant
sp dil	17837	0.14	N/S	sp dil	9953	1.9	N/S
op	19213			op	22030		
sp	30327			sp	9517		
op dil	18821			op dil	12468		
water	25893			water	9984		

2.3 **2.4**

20 day	means/ m^2	Anova $F_{4,12}$	Anova Significant	40 day	means/ m^2	Anova $F_{4,12}$	Anova Significant
sp dil	22706	0.82	N/S	sp dil	21258	0.35	N/S
op	19746			op	23060		
sp	17445			sp	15590		
op dil	15809			op dil	12495		
water	28726			water	20513		

Results of ANOVA based on numbers of collembola found in soil cores
predisposal and at 10, 20 and 40 days after sheep dip disposal at Newton Rigg Site B, 2002

2.5 **2.6**

Pre-disposal	means/ m^2	Anova $F_{4,12}$	Anova Significant	10 day	means/ m^2	Anova $F_{4,12}$	Anova Significant
sp dil	3920	1.10	N/S	sp dil	1629	0.83	N/S
op	3125			op	3908		
sp	7928			sp	2077		
op dil	3976			op dil	4925		
water	10329			water	2667		

2.7

20 day	means/ m^{-}	Anova $F_{4, 16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	13532	**3.54**	Yes	sp dil. vs op	**reject H_0**
op	4119		P<0.05	sp dil. vs sp	**reject H_0**
sp	4086			sp dil. vs op dil.	**reject H_0**
op dil	2827			sp dil. vs water	accept H_0
water	16654			op vs sp	accept H_0
				op vs op dil.	accept H_0
				op vs water	**reject H_0**
				sp vs op dil.	accept H_0
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

2.8

40 day	means/ m^{-}	Anova $F_{4, 12}$	Anova Significant
sp dil	8800	1.28	N/S
op	10427		
sp	4941		
op dil	2060		
water	5412		

Results of ANOVA based on the number of mites found in soil cores predisposal and at 10, 20 and 40 days after sheep dip disposal at Newton Rigg Site B, 2002

2.9

Pre-disposal	means/ m^{2}	Anova $F_{4, 12}$	Anova Significant
sp dil	5547	0.53	N/S
op	6835		
sp	11835		
op dil	7177		
water	7349		

2.10

10 day	means/ m^2	Anova $F_{4, 20}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	4528	**2.89**	Yes	sp dil. vs op	accept H_0
op	5839		P<0.05	sp dil. vs sp	accept H_0
sp	6600			sp dil. vs op dil.	**reject H_0**
op dil	2670			sp dil. vs water	accept H_0
water	4772			op vs sp	accept H_0
				op vs op dil.	**reject H_0**
				op vs water	accept H_0
				sp vs op dil.	**reject H_0**
				sp vs water	accept H_0
				op dil. vs water	**reject H_0**

2.11

20 day	means/ m^2	Anova $F_{4, 16}$	Anova Significant	Tukey test Comparison	conclusion
sp dil	5917	**3.22**	Yes	sp dil. vs op	accept H_0
op	5872		P<0.05	sp dil. vs sp	**reject H_0**
sp	5564			sp dil. vs op dil.	**reject H_0**
op dil	2303			sp dil. vs water	accept H_0
water	7474			op vs sp	accept H_0
				op vs op dil.	**reject H_0**
				op vs water	accept H_0
				sp vs op dil.	**reject H_0**
				sp vs water	**reject H_0**
				op dil. vs water	**reject H_0**

2.12

40 day	means/ m^2	Anova $F_{4, 12}$	Anova Significant
sp dil	6100	1.40	N/S
op	6159		
sp	5444		
op dil	3452		
water	4029		

Appendix 4

General habitat requirements of five species of upland wading birds

Bird Species	General habitat requirements	Nesting requirements	Chick rearing requirements	Out of breeding season
Golden Plover	Unenclosed upland moors and peatlands, above natural tree-line. Terrain over which it can easily run, e.g. no steep slopes or dense vegetation. Raised places for lookouts. Patchy habitat, usually in transition, e.g. where heather burning occurs.	Close to trees, shrubs or low walls. (Glutz et al, 1975)	Areas of increased food resources Shelter Less disturbance	Close-grazed open grassland and farmland of open character. (Fuller & Youngman, 1979)
Lapwing	Moist ground to give ready access to surface and sub-surface inverts. Unenclosed terrain, relatively flat or gently undulating ground, easy to walk on.	Short vegetation	Increased food resources	Some stay within 100km of nesting site, others move further south and west
Snipe	Areas of impeded drainage. Access to shallow water. Tall or dense vegetation separated by open ground, clumps of vegetation as lookout posts. Soft ground for probing.	Dry areas close to wetter zones for feeding	Areas of impeded drainage Access to shallow water	Moves widely to areas of good food supply
Curlew	Wet and dry patches of terrain. Open landscapes with low or sparse vegetation, typically managed moorland.	Rough grass fields Dry nesting sites	Damp feeding areas	Moves to marine env.
Redshank	Moist or wet grasslands. Open or gently sloping ground. Mounds for lookout facilities.	Open areas, only sparse, short, vegetation near wet feeding areas	Moist ground of high invert. biomass availability.	Coastal

(predominantly sourced from Cramp et al, 1983)

Appendix 5

The main foods taken by five species of adult wading birds (predominantly sourced from Cramp et al, 1983)

Invertebrates	Golden Plover	Lapwing	Snipe	Curlew	Redshank
Beetles (adults and larvae)	√	√	√	√	√
Lepidoptera (adults and larvae)	√	√	√	√	
Tipulids (adults and larvae)	√	√	√		√
Other flies	√	√	√	√	√
Other fly larvae	√	√			
Bugs	√	√	√	√	√
Froghoppers	√				
Ants	√	√		√	√
Dragonflies and Mayflies	√	√	√	√	√
Caddisflies and larvae		√	√	√	√
Damselflies		√			
Orthopterans	√	√		√	√
Earwigs	√	√		√	√
Spiders	√	√	√	√	√
Millipedes	√	√			
Snails	√				
Molluscs and Crustaceans	√	√	√	√	√
Harvestmen		√			
Woodlice		√		√	
Slugs	√				
Weevils		√			
Leeches			√		
Earthworms	√	√		√	√
Other worms e.g. Nereids			√		
Small amts. other inverts	√	√	√	√	√
Vertebrates and other food					
Frogs		√	√	√	√
Fish		√		√	√
Vegetation (seeds, grass etc.)	√	√	√	√	√

NB.There is less detailed information about chick diet but it is generally accepted that chicks eat a similar diet to that of the adults, a theory supported by Boyle, 1956, who found that Lapwing chicks less than 2 weeks old can perform the food probe technique.

The table is not intended to be a definitive list of bird foods but an indication of the important food types regularly taken. The opportunistic nature of bird feeding has resulted in different proportions of the invertebrates occurring in different studies on the same species. Different species of invertebrates are consumed at various times of the year due to availability and the dietary requirements of the birds e.g. during the breeding season. This will be discussed further in the main text.

Appendix 6

Golden Plover Chick Foraging Areas

(Whittingham, unpublished data)

WD=Widdybank
CF=Chapel Fell

Site	Brood no.	Potential home range	MCP	Area used with potential home range MCP/Potential
WD	1	55.6	7.28	0.130935252
WD	2	21.7	4.32	0.199078341
WD	3	45.48	2.44	0.053649956
WD	4	4.94	1.88	0.380566802
WD	5	51.36	18.8	0.366043614
WD	6	35.32	2.32	0.065685164
WD	7	14.28	3.84	0.268907563
WD	8	29.24	3.84	0.131326949
WD	9	16.56	4.76	0.287439614
WD	10	146.72	12.12	0.082606325
WD	11	18.08	1.76	0.097345133
CF	1	114.04	6.04	0.052963872
CF	2	113.96	9.8	0.085995086
CF	3	20.44	4.12	0.201565558
CF	4	27.48	3.84	0.139737991
CF	5	21.88	5.72	0.26142596
CF	6	54.88	10.16	0.185131195
CF	7	30.2	3.52	0.116556291
CF	8	96.8	1.64	0.016942149
CF	9	25.44	6.42	0.252358491
CF	10	118.76	7.28	0.061300101
CF	11	84.8	1.4	0.016509434
				Mean % area 0.15700322

MCP are minimum convex polygons, created by joining outer radio locations from radio tracked chicks with a straight line.

Potential Home Range is calculated by drawing a circle of radius around the nest site, radius length being the maximum recorded distance each chick moved from the nest.

(Methodological details in Whittingham et al., 2001)

Appendix 7

The effects of different treatment types on invetbrate prey requirements of chicks

OP, pre - 40 day						SP, 40 day					OP dil, 40 day				
time (days)	Mass (g)	ME (KJ)	Area (ha)	KXF (KJ)	no. of chicks	Mass (g)	ME (KJ)	Area (ha)	KXF (KJ)	no. of chicks	Mass (g)	ME (KJ)	Area (ha)	KXF (KJ)	no. of chicks
1	20	67	0.001	27	0	20	67	0.001	76	1	20	67	0.001	76	1
3	30	97	0.006	239	2	30	97	0.006	686	7	30	97	0.006	681	7
5	40	126	0.017	663	5	40	126	0.017	1905	15	40	126	0.017	1893	15
7	50	154	0.034	1300	8	50	154	0.034	3734	24	50	154	0.034	3710	24
9	60	182	0.057	2149	12	60	182	0.057	6173	34	60	182	0.057	6132	34
11	70	209	0.085	3211	15	70	209	0.085	9221	44	70	209	0.085	9160	44
13	80	236	0.118	4484	19	80	236	0.118	12879	54	80	236	0.118	12794	54
15	90	263	0.157	5970	23	90	263	0.157	17147	65	90	263	0.157	17034	65
17	100	290	0.202	7668	26	100	290	0.202	22024	76	100	290	0.202	21879	76
19	110	316	0.252	9579	30	110	316	0.252	27511	87	110	316	0.252	27330	86
21	120	342	0.308	11702	34	120	342	0.308	33607	98	120	342	0.308	33387	98
23	130	368	0.370	14037	38	130	368	0.370	40314	110	130	368	0.370	40049	109
25	140	394	0.437	16584	42	140	394	0.437	47629	121	140	394	0.437	47316	120
27	150	419	0.473	17937	43	150	419	0.473	51516	123	150	419	0.473	51177	122
29	160	445	0.510	19343	44	160	445	0.510	55555	125	160	445	0.510	55190	124

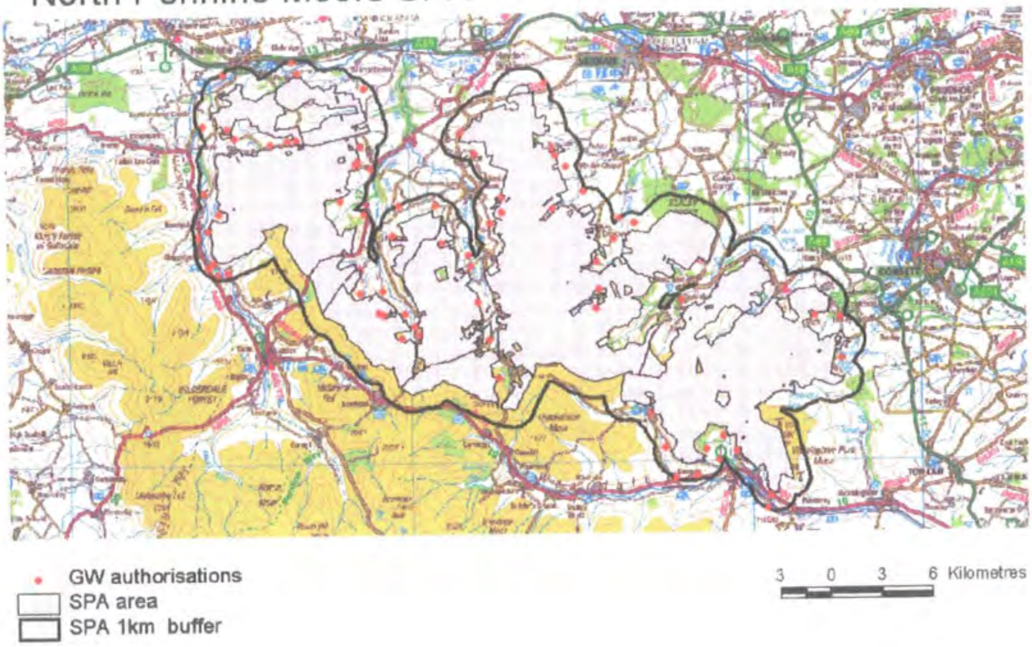
	SP dil, 40 day					Water				
time (days)	Mass (g)	ME (KJ)	Area (ha)	KXF (KJ)	no. of chicks	Mass (g)	ME (KJ)	Area (ha)	KXF (KJ)	no. of chicks
1	20	67	0.001	62	1	20	67	0.001	173	3
3	30	97	0.006	561	6	30	97	0.006	1560	16
5	40	126	0.017	1559	12	40	126	0.017	4333	34
7	50	154	0.034	3056	20	50	154	0.034	8494	55
9	60	182	0.057	5051	28	60	182	0.057	14040	77
11	70	209	0.085	7546	36	70	209	0.085	20974	100
13	80	236	0.118	10539	45	80	236	0.118	29294	124
15	90	263	0.157	14031	53	90	263	0.157	39001	148
17	100	290	0.202	18023	62	100	290	0.202	50095	173
19	110	316	0.252	22513	71	110	316	0.252	62575	198
21	120	342	0.308	27502	80	120	342	0.308	76442	223
23	130	368	0.370	32989	90	130	368	0.370	91696	249
25	140	394	0.437	38976	99	140	394	0.437	108336	275
27	150	419	0.473	42157	101	150	419	0.473	117176	280
29	160	445	0.510	45462	102	160	445	0.510	126363	284

KXF is the energy available to the chicks from invertebrate prey
ME is the energy required by the chicks at a certain mass
Area is the area that the chicks could cover in search of their invertebrate prey requirements
No. of chicks are those that can be supported given the distance they can move at certain mass/age

Appendix 8

Case Study A:

North Pennine Moors SPA



Appendix 9

Experimental exposure of tipulid larvae to dip application

Introduction

The length of time that the dip remains toxic is an important factor in the assessment of detrimental effects of disposal on upland farms. There is evidence that arthropods exposed on plants to pyrethroid application are more at risk than species at ground level (Brown *et al.*, 1988) but it is not clear how much of this difference can be attributed to the greater exposure on the plant and how much to inactivation at the soil surface. *Bembidion lampros* exposed at the soil surface three days after Deltamethrin application and six days after Dimethoate application experienced no mortality Cilgi *et al.* (1988). However, there is evidence that Cypermethrin is more persistent, remaining detectable in the soil as long as seven months after application to control spruce bark beetles (Class, 1992).

Tipulid larvae are an important component in the diet of both adults and chicks of wading birds on upland pastures (Baines, 1990; Galbraith *et al.*, 1993; Whittingham *et al.*, 2001) and these were used in a small preliminary trial on the toxicity and persistence of Diazinon and Cypermethrin applied to soil. The evidence of OP and SP toxicity to tipulids from the historic disposal sites on the farms was not clear. Yorkshire 4 and Teesdale 1 (in 1999), which received multiple applications of dip, showed significant reductions in tipulid larval densities on disposal areas but Teesdale 2, where the disposal area had a high organic content, and Yorkshire 2 had higher densities on disposal sites, compared with control areas. The present trial was designed to investigate whether dip applied to the soil and surface vegetation was toxic to larvae and to determine how long the effect persisted. The trial was not part of the original research contract but is reported here as relevant to the interpretation of the field study.

Methods

The main constraint on this toxicity trial was the sampling and extraction time required to collect an adequate supply of *Tipula subnodicornis* larvae from the field. Seventy larvae only were collected. Because there was no background knowledge of the length of time Diazinon or Cypermethrin might remain toxic to tipulids, it was decided to use these larvae to establish the appropriate time span for a study of persistent effects. In each trial six replicates only were used. This did not allow reliable LD_{50} estimates to be made but it did allow the larvae to be exposed to dip at a series of five time intervals from initial application.

Twelve cultures of leafy liverworts (Butterfield, 1976), in ericaceous compost were set up in 10 cm diameter plastic plant pots on 13 July and 42 cultures on 31 October. Diazinon and Cypermethrin were applied to six pots each on 13 July and to 18 pots each on 31 October. Each pot received 100 ml of sheep dip applied at the disposal dilution for made up dip, equivalent to the full strength application at Sourhope. Six control cultures without dip application were also set up on 31 October and received 100 ml of water.

One *Tipula subnodicornis* larva was introduced to each of six Diazinon, six Cypermethrin and six control cultures on 31 October (day 0 after application). The top of each plant pot was covered by polythene secured with an elastic band and the pots were placed in plant trays outside. Six further Diazinon and Cypermethrin cultures set up on 31 October received a larva each on 1 November (day 1 after application) and on 7 November (day 8 after application). On 9 November, some of the larvae were not found in the cultures, so compost and liverworts were transferred from each of six Cypermethrin, six Diazinon and six control pots set up on 31 October to 50 ml screw-top vials. On 16 November (day 17 after application), one larva was placed in each vial. On 25 November (19 weeks after application) compost and liverworts were transferred to vials from the six Diazinon and Cypermethrin pots set up in July and one larva placed in each vial.

Results

Table 1: Percentage mortality of *Tipula subnodicornis* larvae exposed to Diazinon and Cypermethrin (at standard dip dilution) at different time periods after application. Probabilities in brackets represent comparisons between treatment and control based on Fisher's Exact Test.

Days after application	% mortality in treatment: Cypermethrin	Diazinon	Control
0	100 (p=0.005)	100 (p=0.005)	0
1	100 (p=0.005)	100 (p=0.005)	0
8	67* (p=0.05)	83* (p=0.01)	0
17	50 n.s.	83 (p=0.01)	0
133	33 n.s.	67 n.s.	0

* One larva not found

Tipulid larvae which were exposed on the day of application, or the day after, to either Diazinon or Cypermethrin, all died within two days (Table 1). There was no mortality in the controls (for each of the four comparisons with the controls $p = 0.005$, Fisher Exact Test). When larvae were introduced eight days after the dip application, mortality was not instantaneous in most cases and the disappearance of six of the larvae from the cultures indicated an active response in some individuals. All but one of the missing larvae in each set of dip cultures were found dead in the tray containing the plant pots, seven days after the larvae were introduced. One larva survived 14 days in the Cypermethrin cultures but no larvae survived in Diazinon, giving significant differences from the controls in both cases ($p = 0.05$ and $p = 0.01$ respectively, Fisher Exact Test). Larvae introduced to the cultures 17 days after application of Diazinon suffered significant mortality ($p = 0.01$, Fisher Exact Test) but not 17 days after the application of Cypermethrin. Even after 133 days, the cultures where dip had been applied appeared to retain some toxicity with four larvae dying in the Diazinon culture and two in Cypermethrin over a two week period (pooling dip cultures ($N = 12$) and comparing with the six controls, $p < 0.05$, Fisher Exact Test). The surviving larvae, and those remaining from the cultures set up on 16 November, were all alive 41 days later on 5 January 2001 but, in comparison with the six control larvae, the three larvae in the Diazinon cultures were unresponsive and thin with no fat reserves.

Discussion

These toxicity trials were carried out on small numbers of larvae and obviously need to be verified by being repeated on a larger scale and under experimental conditions that are more closely equivalent to the natural situation. In particular, the experiment should be repeated using larvae that have been allowed to establish themselves in burrows in grass turves before the insecticide application is made. It was not anticipated that larvae would escape from flowerpots, as the negatively phototactic, third instar larvae of *Tipula paludosa* can be reared in open boxes (Szewczyk and Langenbrush, 1997). The small containers used for the 17 and 133 day trials did not have drainage holes and this may have contributed to the maintenance of high toxicity levels in the compost. Despite these reservations, the results suggest that both dips may remain toxic to soil invertebrates over longer periods than anticipated (Cilgi *et al.*, 1988). The apparent lingering toxicity of Diazinon 19 weeks after application is particularly surprising and needs to be verified under more rigorous conditions. The mini-trial also suggested that the tipulids might have been behaving atypically after receiving the dip application, coming to the surface and moving out of the plant pots, before dying. Under field conditions, and if birds are present on the pastures, surface activity of dying tipulids could lead to contaminated individuals being eaten. The present study was not concerned with the direct effects of insecticide-contaminated prey poisoning birds. However, a decrease of 64% in cholinesterase activity was observed in shrews when they were fed earthworms which had been released into dimethoate treated soil (ChE activity in the worms was depressed by 90%), one day after application (Dell'Omo *et al.*, 1999). The possibility of behavioural changes, following dip application, that make the invertebrate prey more attractive to birds needs further investigation.

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